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ABSTRACTS

CONTENTS

The Tito Bastianello Awards pages 2 - 8
Oral Presentations pages 9 - 26
Poster Pitch Presentations pages 27 - 54
E-Poster Viewing pages 55 - 146
Invited Speakers pages 147 - 162
Tito Bastianello Awards

001 / #97

Topic: AS04-MDS Biology and Pathogenesis / AS04d-Somatic mutations

The Tito Bastianello Award

THE TITO BASTIANELLO AND MDSF YOUNG INVESTIGATORS AWARDS
09-26-2021 12:30 PM - 1:30 PM

TRAJECTORIES OF MDS EVOLUTION IN THE CONTEXT OF APLASTIC ANEMIA

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Background And Aims: Clonal evolution occurring in about 20% of aplastic anemia (AA) patients provides instructive clues for the general understanding of MDS.

Methods: An integrative analysis of germline (GL) and somatic leukemogenic and immune-escape mutations (HLA,PIGA) was performed during AA to MDS progression.

Results:

Among 350 AA patients, 11% developed MDS at a median time of 6yrs. MDS evolution was less common in moderate AA and reciprocal to PNH clone expansion (p=.0003) with abnormal karyotype present in...
83% of patients, involving chr.7 in 47% of cases (vs. 7.5% in primary MDS-pMDS, Fig.1A-B). GL pathogenic (e.g., SAMD9L, SBDS) alterations (n=9) and likely-pathogenic (n=11), were enriched in progressors and del(7q) patients (p=.0001) but no immunogenetic associations (HLA risk-alleles nor evolutionary divergence) were found. Progression was associated with acquisition of new somatic hits, including e.g., ASXL1, SETBP1 and ETV6 in del(7q)/-7 carriers (Fig.1C-F). In 9 patients antecedent TET2, DNMT3A and ASXL1 mutant clones were present and further expanded upon MDS evolution (p=.0001; Fig.1G-H). Clonal hierarchy by allelic imbalance identified del(7q)/-7 as a founder lesion in 60% of patients (vs. 50% in pMDS). Concomitant with leukemogenic mutations, 21% of progressors (vs. 13% of non-progressing AA; Fig.1I) harbored somatic HLA class-I/II lesions, while PIGA clones were found in 9% of cases.

Conclusions: In the context of immune-mediated attack and common immunogenetic predisposition, clonal evolution is a maladaptive phenomenon characterized by acquisition of immune-escape phenotype (HLA,PIGA mutations) and somatic leukemogenic events. In addition to complete polyclonal hematopoietic recovery, evolution of HLA-mutant or PIGA/PNH hematopoiesis corresponds to adaptive and semi-adaptive clonal recovery, respectively.
A PRE-CLINICAL PATIENT-DERIVED XENOGRAFT MODEL OF MYELODYSPLASTIC SYNDROMES DEMONSTRATES EFFICACY AND SAFETY OF ELTROMBOPAG

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Background And Aims: Pre-clinical research of MDS is impeded by the lack of feasible in-vitro and in-vivo models. Previously, we have established a robust niche-based PDX model of MDS. In this study, we demonstrate for the first time that this model is applicable as a pre-clinical platform to address pending clinical questions by investigating the efficacy and safety of the thrombopoietin receptor agonist eltrombopag (EPAG).

Methods: BM derived CD34+ cells and MSCs of MDS patients were intrafemorally transplanted (IF TX) into NSG mice. Engrafted mice were treated with EPAG or vehicle for 18-24 weeks. In PB, huCD41+ platelets were absolutely quantified using a bead-based flow cytometric assay. Sorted huCD45+ BM cells of PDX samples at different time points were analyzed by whole exome sequencing to determine clonal evolution throughout treatment.

Results: In total, we included n=49 PDX of n=9 MDS primary patient samples. In EPAG-treated PDX, overall response rate in human platelets was 56%. Increase in human megakaryocytes was confirmed by IHC and FACS. Human engraftment and percentage of huCD34+ cells were not affected by EPAG treatment. The clonal BM composition remained stable in the majority of cases. Novel molecular lesions were observed in n=4 (17%) vehicle- and n=2 (8%) EPAG-treated PDX.

Conclusions: In conclusion, this experimental setup allowed for vehicle- and replicate-controlled analyses on a patient-individual level deciphering substance-specific effects from natural disease progression thereby supporting efficacy and safety data of EPAG in MDS, and validating our MDS PDX model to be a useful tool for testing new therapeutic concepts in MDS preceding clinical...
trials.
Background And Aims: Curative potential of allogeneic hematopoietic stem cell transplantation (HSCT) in myeloid malignancies depends on effective graft versus leukemia effect (GvL). Decreased expression or genomic aberrations involving human leukocyte antigen (HLA) region (somatic 6p deletion/loss of heterozygosity [LOH]) have been described in haploidentical/mismatched and matched contexts as mechanisms facilitating leukemic relapse. We hypothesized that somatic mutations in class I-II HLA alleles may also contribute to immune escape from GvL.

Methods: We performed a genetic characterization of sequential specimens from 48 patients with MDS and AML relapsing after HSCT, assessing HLA region along with 173 leukemia-associated genes.
Results:

Ninety-six serial samples (39 at MDS/AML diagnosis, 48 at post-HSCT and 9 at post-chemotherapy relapse) were analyzed. Disruptive HLA mutations were found in 29% of the patients (4% at diagnosis and 25% at post-transplant relapse), in both class I and II loci. In post-transplant group, 75% of those events were found in patients receiving graft from a matched donor, while 25% received a haploidentical transplant. Patients with HLA mutations had more likely a later relapse (median time: 554 vs 150 days after transplant, p=.00042). Also, among those who received donor lymphocyte infusion-based regimens, none of the HLA-mutated (0/6) vs 12/19 HLA wild-type patients had transient or stable responses.

Interestingly, when examining the somatic myeloid landscape, HLA-mutated cases were enriched in genetic aberrations in epigenetic regulators (such as TET2, EZH2, EP300 and DNMT3A) (Figure 1).

Conclusions: In conclusion, here we describe the existence of a new mechanism of HLA-loss and post-transplant immune escape facilitating leukemia relapse.
Background And Aims: SF3B1 mutations (SF3B1MT) cause abnormal erythroid terminal differentiation, resulting in myelodysplastic syndromes with ringed sideroblasts (MDS-RS), which are characterized by peripheral blood (PB) anemia and the accumulation of ringed sideroblasts in the bone marrow (BM). Many studies have analyzed the transcriptional and splicing landscape of bulk hematopoietic stem and progenitor cells (HSPCs) with SF3B1MT. Here, we sought to understand how SF3B1MT affect the differentiation potential of HSPCs and more differentiated BM mononuclear cells (MNCs) at the single-cell level.

Methods: We used single cell technologies combined with functional studies to analyze the expression profile of single HSPCs and MNCs isolated from patients with MDS-RS at different stages of the disease.

Results: We found that SF3B1MT enhance an initial differentiation of MDS stem cells towards the erythroid lineage but then induce a more downstream differentiation arrest when erythroid cells are at the orthochromatric step of maturation before their release into the PB. Expression analysis of erythroid cells across the last stages of erythroid differentiation in the BM revealed a significant upregulation of genes involved in the response of eukaryotic translation initiation factor 2 alpha kinase 1 (EIF2AK1) to heme deficiency, including EIF2AK1, EIF2S1, ATF4, and DDIT3, culminating in the upregulation of the autophagy signature in orthochromatic cells. These aberrant pathways were completely rescued when patients achieved complete remission and became transfusion-independent after hypomethylating agent treatment.

Conclusions: Our findings suggest that the EIF2AK1 signaling pathway should be further investigated to help develop therapies that elicit stable responses in MDS-RS patients.
Oral Presentations

O05 / #138

Topic: AS04-MDS Biology and Pathogenesis / AS04b-Clonal diversity & evolution

PLENARY SESSION: BM MICROENVIRONMENT AND INFLAMMATION
09-23-2021 3:45 PM - 5:40 PM

SOMATIC GENETIC RESCUE IN SAMD9/SAMD9L MDS PREDISPOSITION SYNDROMES


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Background And Aims: SAMD9 and SAMD9L (SAMD9/9L) are novel MDS predisposing genes associated with monosomy 7. We studied a population-based cohort of 669 children and adolescents with MDS enrolled over 20 years to describe prevalence, clinical outcome, and interrogate phenotypic
associations and functional consequences of \textit{SAMD9/9L} mutations (\textit{SAMD9/9L}\textsubscript{mut}) compared with germline \textit{GATA2} mutations (\textit{GATA2}\textsubscript{mut}).

\textbf{Methods:} Clinical phenotyping, bulk, and single-cell genomics, \textit{in-silico} scoring, HEK293 cell assays (Fig.1)

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Diagram illustrating the study design and results.}
\end{figure}

\textbf{Results:} Mutually exclusive germline \textit{SAMD9/9L}\textsubscript{mut} or \textit{GATA2}\textsubscript{mut} were detected in 8\% or 7\% of consecutive pediatric MDS, and 21\% or 30\% of monosomy 7 subgroup. Germline \textit{SAMD9/9L}\textsubscript{mut} predominantly associated with refractory cytopenia of childhood and did not negatively affect prognosis and outcome after hematopoietic stem-cell transplantation. In total, we identified 67 patients with 58 germline \textit{SAMD9/9L}\textsubscript{mut} clustering to protein middle region (>90\% causing growth arrest in HEK293 cells) and 16 somatic \textit{SAMD9/9L}\textsubscript{mut}. Using a population genomics approach, we discovered \textit{SAMD9/9L} disease-specific phenotypes to correlate with germline mutations that are either absent or ultra-rare (<0.005\%) in population. Bulk and single-cell DNA sequencing revealed somatic genetic rescue (SGR) events causing loss or inactivation of germline \textit{SAMD9/9L}\textsubscript{mut} in 61\% of patients. Among those, half (51\%) had SGR clones with adaptive potential (revertant UPD7q, somatic \textit{SAMD9/9L}\textsubscript{mut}), while 95\% had maladaptive SGR (monosomy 7 alone or with cancer mutations). The discovery of multiple competing SGR events in single patients highlights the strong negative selective pressure of \textit{SAMD9/9L}\textsubscript{mut}.

\textbf{Conclusions:} \textit{SAMD9/9L}\textsubscript{mut} cause a predisposition syndrome with highest rates of rescue clonal hematopoiesis discovered in humans and exemplify the extreme plasticity of hematopoietic system early in life.
MIR99B AND MIR125A AS NONINVASIVE PROGNOSTIC PLASMA BIOMARKERS IN MDS

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Background And Aims: The diagnosis of MDS is based on invasive cytomorphological analysis of peripheral blood and bone marrow cells. Much research has been conducted on the molecular mechanisms and epigenetic pathways in MDS and their prognostic and therapeutic significance, but few studies on the importance of miRNA in MDS.

Methods: Levels of selected miRNAs were determined in plasma samples of 20 healthy volunteers and 41 untreated MDS patients diagnosed at the Department of Hematology at Merkur University Hospital. Gene expression of specific miRNAs hsa-miR-125a, hsa-miR-99b, were determined by real-time polymerase chain reaction (Quantitative Real Time Polymerase Chain Reaction - q-RT PCR) according to the manufacturer’s protocol. Relative expression levels were calculated by 2-ΔΔCT method.

Results: Significant differences between the tested group and healthy control level of miR-99b show an increased level in the subjects compared to the control by 4,521 times (P = 0.004). There were significant negative correlations between the level of miR-125a and the number of RBCs and hemoglobin. miR-99b significantly positively correlated with WBC count. The highest mean level of miR-99b and miR-125a were in the group of higher-risk MDS patients.

Conclusions: Despite the limitation of the study with small numbers of MDS patients, these results suggest the possible importance of miR-99b as a diagnostic marker in MDS, while both miR-99b and miR-125a could serve as valuable noninvasive prognostic parameters. Given recent studies on a noninvasive diagnostic model in MDS, circulating miRNA may be an additional parameter that will contribute to the greater sensitivity and specificity of noninvasive diagnostic tests.
COMBINED LANDSCAPE OF SINGLE-NUCLEOTIDE VARIANTS AND COPY-NUMBER ALTERATIONS IN CLONAL HEMATOPOIESIS: ANALYSIS IN 11,234 JAPANESE INDIVIDUALS

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Background And Aims: Clonal hematopoiesis (CH) in apparently healthy individuals has recently been highlighted and implicated in the development of hematological malignancies (HM) and cardiovascular mortality. In previous studies, CH has been investigated through detecting either single-nucleotide variants and indels (SNVs/indels) or copy number alterations (CNAs), but not both.

Methods: Here, we performed a combined analysis of CH-related SNVs/indels and CNAs in 11,234 individuals aged ≥60 years (a median of 70), including 672 with subsequent HM events, to delineate their relationships and impacts on clinical outcomes.

Results: Detected in 40% of the subjects, CH-related SNVs/indels and/or CNAs were significantly cooccurred in the same individuals. CH-related SNVs/indels and CNAs coordinately affected blood cell counts and the mortality from HM, depending on the maximum clone size and total number of both lesions (Figure1). In particular, as is the case with those in myeloid neoplasms, SNVs/indels and CNAs affecting DNMT3A, TET2, JAK2, and TP53 significantly co-occurred and led to bi-allelic alterations in these genes in the early leukemogenesis, predicting a high HM mortality. CH-related SNV/indels were also associated with the risks of hypertension and cardiovascular mortality. This association was more prominent when SNVs/indels were combined with CNAs, even though cardiovascular mortality was not...
affected by CNAs alone (Figure 2).

**Conclusions**: These findings provided novel insight into the interplay between SNVs/indels and CNAs in CH, highlighting the importance of detecting both types of lesions in the evaluation of CH.
LYMPHOPENIA IS HIGHLY PREVALENT IN MDS AND PROVIDES ADDITIONAL PROGNOSTIC INFORMATION FOR IPSS-R VERY-LOW AND LOW-RISK PATIENTS. AN ANALYSIS FROM THE EU-MDS REGISTRY


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Background And Aims: In MDS the clinical course is primarily determined by the extent of myeloid insufficiency and the risk of AML-evolution. Data on the role of an impaired lymphopoiesis are emerging.

Methods: The database of the EU-MDS registry was screened for patients with information about the absolute lymphocyte count (ALC) at diagnosis and during follow-up. Lymphopenia was defined as an ALC < 1.2 x 10⁹/µl. Cases with an ALC ≥ 5.0 x 10⁹/µl were excluded.

Results: 2377 patients were identified (62% male, 38% female; median age 74 years, median follow-up 44.2 months), of whom 1469 were lymphopenic (970 at diagnosis, 499 during the observational period). Lymphopenic patients had lower platelet and neutrophil counts (median 142 vs. 198 x 10⁹/µl and 1.9 vs. 2.7 x 10⁹/µl, p < 0.001 each) and were more frequently transfusion-dependent (34 vs. 26%, p < 0.001). Between the IPSS-R cytogenetic risk categories, no difference was noted with regard to the proportion of lymphopenic patients. Age-adjusted median survival was shorter for patients with an ALC < 1.2 x 10⁹/µl (101.3 versus 54.0 months, p < 0.001). No difference was noted for IPSS-R-very-high-, high- and intermediate-risk patients (n = 25, 85 and 347, respectively). For very-low (n = 1240) and low-risk patients (n = 1425), lymphopenia was associated with a shorter survival (91.5 vs. 134.4 months, p < 0.001, and 52.8 versus 79 months, p = 0.001, respectively).
**Conclusions:** Our data confirm the relatively high prevalence of lymphopenia in MDS patients. For IPSS-R-very low and low-risk patients, lymphopenia provides additional prognostic information. Further research will focus on the influence of the mutational status as determined by NGS on the ALC.
Mutations were more frequent in MDS (5.1%) and secondary AML (7.6%) than primary AML (1.4%). The

Results: We identified DDX41 mutations in a total of 221 (3.9%) patients. Germline mutations were found in 189 (86%) cases, of which 118 had somatic mutations, while 32 (14%) had somatic mutations alone. Mutations were more frequent in MDS (5.1%) and secondary AML (7.6%) than primary AML (1.4%).

Background And Aims: DDX41 is one of the most frequent targets of germline mutations found in myeloid neoplasms (MN). In this study, we aimed to elucidate a full spectrum of DDX41 mutations and associated clinical pictures.

Methods: MN-related mutations were investigated in a total of 5,617 sporadic cases with MN using targeted-capture sequencing.
majority of germline mutations (124/189; 66%) were truncating mutations, which, however, were rarely (1.9%) found in somatic mutations. Germline mutations showed an ethnic diversity. For example, M11 and A500fs were specific to European and Asian populations, respectively. Many germline mutations were shown to be significantly enriched in MN, albeit with different odds ratios; A500fs (12.1), E7X (11.0), Y259C (14.3) and E256K (7.81). Compared with unmutated patients, DDX41-mutated cases showed a significantly lower mutation frequency of NRAS and TET2 mutations, while a higher frequency of CUX1 mutations. In addition, DDX41-mutated cases, particularly when treated with hypomethylating agents (HMAs), had a better OS than un-mutated cases, even though showing more rapid AML progression ($P=0.0063$). In particular, AML progression in HMA-treated DDX41-mutated cases was comparable to that in HMA-treated unmutated cases.

**Conclusions:** *DDX41*-mutated MN is a unique entity of MN associated with unique genetic feature and a favorable OS, particularly when treated with HMA.
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Background And Aims: Majority of MDS patients suffer with coronary artery disease (CAD). Anaemia and thrombocytopenia pose unique challenges for the management of CAD due to increased bleeding risk. We aim to assess burden and management of acute CAD in MDS.

Methods: Medical records of cardiac hospitalisations were analysed in 910 patients registered in the South Australian MDS registry.

Results: During median follow-up of 28.5 (95% CI 10.8-68.6) months, 274 (30%) patients required 336 hospitalisations for cardiac causes. Complete data were available for 84 of the 91 hospitalisations for CAD, of which 51 (60.7%) were due to Type 1 myocardial infarction (MI), consisting of 38 cases of non-ST elevation MI (NSTEMI) and 7 of ST-elevation MI (STEMI). Overall, 43 out of the total 910 patients (5%) had an admission for Type 1 MI with 8 of these having more than one MI during study period. Coronary angiography was performed in only 37% of cases of Type 1 MI and percutaneous coronary intervention in 20%. Meanwhile, all guideline-recommended pharmacotherapies were under-prescribed at discharge: 78% use of antiplatelets, 62% statins, 51% beta-blockers, 38% ACE inhibitors/angiotensin receptor blockers. 25% patients had bleeding during or within 6-months of index admission for Type I MI. Re-admission with CAD was 18% following type I
Conclusions: Our data reveal an alarming tendency to manage patients with MI conservatively, with under-utilisation of all guideline-recommended therapies often in the absence of absolute contraindications. This highlights an unmet need for developing guidelines to manage cardiac co-morbidities in MDS.
IS RACE IMPORTANT IN GENOMIC CLASSIFICATION OF HEMATOLOGICAL NEOPLASMS?

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Background And Aims: In recent years, genome-based classifications for hematological neoplasms have been proposed successively and proved to be more accurate than histologic classifications. However, some previous studies have reported the racial differences of genetic landscape in persons with hematological neoplasms including myelodysplastic syndromes (MDS), which may cause a genomic classification based on a particular ethnic group does not operate in others.

Methods: To determine whether race plays an important role in the genomic-based classification, we validated a newly proposed genomic classification of MDS (J Clin Oncol.2021;JCO2001659), which was based on an European database, in Chinese patients from our center.

Results: The genetic profiles of Chinese patients are different from that of Western patients from European cohort, which is consistent with prior studies. These racial heterogeneities consequently led to significant differences between the two populations regarding the proportion of each subgroup to overall cohort when applying this novel genomic classification, for only 16% of our subjects were into cohorts 1, 3-6 defined by most commonly mutated class of genes in MDS that encode splicing factors compared with 67% in Europeans whereas more than ½ of our subjects were in cohort 0 without specific genomic
features compared with 16% of Europeans (P < 0.001).

Conclusions: Our results indicate that a genome-based classification of MDS operating in populations of European descent may not operate in other populations such as Chinese. Given the prevalent racial differences of genetic profiles in hematological malignancies, we believe that it is important to consider race when applying a genomic classification of hematological neoplasms.
O12 / #202

**Topic:** AS06-Prognosis / AS06a-Prognostic factors of outcome and risk assessment

**Plenary Session**

**PLENARY SESSION: CMML AND THE SPLICEOSOME**
09-25-2021 4:45 PM - 6:00 PM

**CLINICAL OUTCOMES AND MOLECULAR PROFILE OF OLIGOMONOCYTIC CHRONIC MYELOMONOCYTIC LEUKEMIA SUPPORT ITS CONSIDERATION AS THE FIRST STEP IN THE PROLIFERATIVE CONTINUUM OF CHRONIC MYELOMONOCYTIC LEUKEMIA**

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**Background And Aims: INTRODUCTION** Recent studies show that OM-CMML and CMML have a similar clinical and biological profile. Since the proliferative-CMML (P-CMML) is the last-stage of CMML proliferative continuum and shows poor outcomes. It is expected that OM-CMML shows the best outcomes of these entities, since it represents an early-stage of dysplastic-CMML (D-CMML). **AIM** To analyze survival outcome data of OM-CMML

**Methods:** Compare 41 OM-CMML to 162 CMML patients (121 D-CMML and 41 P-CMML).
Results:

RESULTS OM-CMML showed longer OS than D-CMML and P-CMML (Figure 1). OM-CMML also showed longer AML-free survival than D-CMML (median OS: 131.8m vs. 43.47m; P=0.001) and P-CMML (median OS: 23m; P<0.01). These outcomes were retained after multivariate adjustment by CPSS (HR: 0.38, 95% CI 0.21-0.70, P=0.002; HR: 2.53, 95% CI 1.64-3.91, P<0.001), CPSS-P (HR: 0.42, 95% CI 0.23-0.78, P=0.005; HR: 2.82, 95% CI 1.92-4.14, P<0.001), and Mayo-prognostic-model (HR: 0.41, 95% CI 0.23-0.75, P=0.04; HR: 3.45, 95% CI 2.29-5.20, P<0.001). At a median follow-up of 45 months, 29% of D-CMML evolved to P-CMML. There was no difference in OS between D-CMML that evolved to those that did not. Nevertheless from the moment of evolution, they had a very short survival (median OS: 10.2 months). This suggests that P-CMML evolution is the latest stage of a biological continuum that initiates in OM-CMML. Reinforcing this idea, mutations associated with proliferation (i.e.: ASXL1 and RAS-pathway) were identified as independent prognostic factors for OS in our series (HR ASXL1: 2.47, 95% CI 1.13-5.37, P=0.023; HR RAS-pathway: 3.91, 95% CI 1.74-8.77, P=0.001).

Conclusions: CONCLUSION The clinical outcomes of OM-CMML support its consideration as the first step in the proliferative continuum of CMML.
WHOLE TRANSCRIPTOME ANALYSIS IDENTIFIES DISTINCT GENE EXPRESSION PROFILES BETWEEN SF3B1MUT AND SF3B1WT MYELODYSPLASTIC SYNDROME WITH RING SIDEROBLASTS

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Background And Aims: The 2016 revised WHO classification incorporated somatic mutation in SF3B1 spliceosome gene within the diagnostic criteria of myelodysplastic syndrome (MDS) with ring sideroblasts (RS). However, SF3B1wt MDS-RS display significantly different clinical features and outcome from those of SF3B1mut MDS-RS. Recently, the recognition of SF3B1-mutant MDS as a distinct nosologic entity has been proposed to overcome this limitation.

Methods: To further validate this proposal, we studied a cohort of 124 MDS patients by whole transcriptome analysis of CD34+ bone marrow mononuclear cells and explored differential gene expression according to morphology and molecular genetics. We restricted our analysis to MDS with bone marrow blasts below 5% and investigated MDS with RS >5% (cases) or MDS negative for both splicing mutation and RS (controls).

Results: SF3B1 mutation was found to be mutually exclusive with SRSF2 and TP53 mutations (q-value <0.05). Therefore, the study population was divided into three categories, MDS-RS-SF3B1mut (n=64), MDS-RS-SF3B1wt (n=25) and MDS-SLD/MLD (n=35). We identified 1566 differentially expressed genes (DEG) between MDS-RS-SF3B1mut and MDS-RS-SF3B1wt and confirmed that ABCB7 downregulation is associated with SF3B1 mutation and not RS phenotype per se (Figure 1AB).
Finally, we identified two clusters of DEG in MDS-RS-\textit{SF3B1}^{mut} and MDS-RS-\textit{SF3B1}^{wt}. K-means
clustering analysis recognized MDS-RS-SF3B\textsuperscript{mut} from other subgroups with 81.1% accuracy. MDS-RS-SF3B\textsuperscript{mut} exhibited a specific downregulation of cellular adhesion and an upregulation of G-alpha signaling molecules (adjusted p-value < 0.01, Figure 2).

**Conclusions:** Overall, this study contributes to unveil molecular features of SF3B\textsuperscript{1}-mutant MDS and provides further evidence to support recognition of somatic SF3B\textsuperscript{1} mutation as a disease-defining genetic lesion.
Poster Pitch Presentations

O14 / #98

Topic: AS04-MDS Biology and Pathogenesis / AS04f-Gene expression profiling

Plenary Session

PLENARY SESSION: LOW RISK MDS
09-24-2021 12:30 PM - 1:55 PM

TET2 DYSFUNCTIONS UNVEIL CLINICAL AND PROGNOSTIC PHENOTYPES IN PATIENTS WITH MDS

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Background And Aims: TET2 gene is involved in regulation of hematopoietic stem cell fate and monocytic commitment via modulation of methylation in promoters of lineage-specific transcription factors. The general down-regulation of 5-hydroxymethylcytosine (5-hmC) argues for a role of DNA demethylation in MDS beyond TET2 mutations, which, albeit frequent, do not convey any prognostic significance. We investigated TETs expression to identify factors, which can modulate the impact of mutations and thus 5-hmC levels on clinical phenotypes and prognosis of MDS patients.

Methods: DNA/RNA-sequencing and 5-hmC data were collected from 1665 MDS patients and 91 healthy controls (HC).
Results:

Irrespective of mutations, a significant fraction of MDS patients exhibited lower TET2 expression, while 5-hmC levels were not uniformly decreased (Fig.1A-B). In searching for factors explaining compensatory mechanisms, we discovered that TET3 was up-regulated in MDS and inversely correlated with TET2 expression in wild-type cases (Fig.1C). While TET2 was reduced across all age-groups, TET3 levels were increased in a likely feedback mechanism induced by TET2 dysfunction. The inverse relationship of TET2 and TET3 expression also paralleled the level of expression of L-2-hydroxyglutarate dehydrogenase, an enzyme involved in agonist/antagonist substrate metabolism (Fig.1D-E). Importantly, elevated TET3 levels influenced the clinical phenotypes of TET2-deficiency in both univariate and multivariate settings whereby the lack of compensation by TET3 was associated with higher risk features (Fig.1F-G).

Conclusions: While TET2 mutations lack prognostic impact, TET2/TET3 expression profiles unveil clinico-prognostic significance in MDS. Application of new therapeutic approaches (e.g., vitamin C) should be informed by analyses of these factors.
BONE MARROW DERIVED STROMAL CELLS FROM MYELODYSPLASTIC SYNDROMES ARE ALTERED BUT NOT CLONALLY MUTATED IN VIVO

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Background And Aims: Increasing evidence suggests that the pathogenesis of Myelodysplastic Syndromes (MDS) is not only driven by the hematopoietic compartment but also the bone marrow (BM) microenvironment. Seminal studies in murine models have demonstrated that isolated mutations in the BM niche can disrupt the non-mutated hematopoietic compartment and induce MDS-like diseases. An unanswered question has therefore been whether primary MDS in humans is possibly also associated with acquired mutations in the BM stroma.

Methods: In order to address this hypothesis, we performed a comprehensive whole exome sequencing study on BM derived in vitro expanded mesenchymal stromal cells (MSCs) of n=98 MDS and myeloid neoplasia patients and a control group of n=20 healthy individuals. In addition, MSCs in serial culture passages and different BM aspirations from the same patients were sequenced in order to investigate whether acquired mutations in MSCs were artefacts of the ex vivo expansion. Putatively high confidence mutations were further backtracked by deep re-sequencing in primary sorted CD45-CD235a-CD31-CD271+ BM cells in n=9 cases.

Results: We discovered multiple recurrently acquired mutations in expanded MSCs of MDS patients in genes such as ZFX (n=8/98), RANK (n=5/98), and others. Expanded MSCs from MDS patients displayed a higher mutational burden and distinct mutational signatures. However, all validation experiments in serial cultures and primary sorted MSCs indicated that the discovered mutations were expanded by in vitro culture but not present in relevant cell populations in the primary BM.

Conclusions: Thus, we conclude that there is no evidence for clonal mutations in the BM stroma of MDS patients.
USING NEXT-GENERATION SEQUENCING TO ASSESS THE NEED FOR BONE MARROW BIOPSIES IN PATIENTS WITH CYTOPENIA

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Background And Aims: Bone marrow biopsies are currently the core of the diagnostic work-up of persistent cytopenias. Aiming to explore whether next-generation sequencing (NGS) could obviate the need for a bone marrow biopsy, we incorporated NGS in a model to predict presence of disease in the bone marrow of patients with cytopenia.

Methods: We analyzed the occurrence of mutations in 508 patients with cytopenias from 2015-2020, referred for primary work-up for a suspected hematological malignancy. We divided patients into a discovery (n = 340) and validation (n = 168) cohort, and NGS of genes associated with myeloid malignancies, bone marrow biopsy and complete blood count (CBC) were performed in all patients.

Results: Mutations were identified in 270 (53%) patients. Abnormal bone marrow morphology was found in 188 (37%) patients. Using logistic regression, we constructed a model to predict the presence of abnormal bone marrow morphology based on age, sex, CBC and mutational status. Mutations in TET2, SF3B1, U2AF1, TP53 and RUNX1 were significantly associated with abnormal bone marrow morphology. In the validation cohort, 28 (17%) patients had less than 10% predicted risk of abnormal bone marrow morphology. None of these patients had abnormal bone marrow morphology, corresponding to a sensitivity of 100% (95% CI: 0.93-1.00). The negative predictive value of having less than 10% risk of abnormal bone marrow morphology was 100% (95% CI: 0.88-
**Conclusions:** This is the first study to show that NGS can be used to assess the necessity of bone marrow biopsies, which can potentially spare patients a painful and unnecessary procedure.
LOW-RISK MYELODYSPLASTIC SYNDROMES (MDS) WITHOUT MUTATIONS ARE AS GOOD AS IDIOPATHIC CYTOPENIA OF UNDETERMINED SIGNIFICANCE (ICUS)

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Background And Aims: Idiopathic cytopenia of undetermined significance (ICUS) and low-risk myelodysplastic syndromes (MDS) are distinguished mainly by morphologic dysplasia, which sometimes shows discrepancy among examiners. We hypothesized that gene mutations are an independent prognostic factor for ICUS and low-risk MDS.

Methods: From four medical centers, we enrolled patients with cytopenia ≥1 lineage (ANC<1,800/mm³, hemoglobin<13 g/dL (male) or<12 g/dL (female), platelet<150x10⁹/mL). Bone marrow examinations were evaluated by hematologists. Diagnosis of low-risk MDS was made according to WHO classification 2016 and revised-international prognostic scoring system (R-IPSS)≤3.5. DNA was extracted from bone marrow/blood and sequenced by targeted next generation sequencing (NGS).

Results: One hundred twenty-three patients were recruited; 25.2% ICUS and 74.8% low-risk MDS. Patients with ICUS were younger than low-risk MDS (68 y.o. vs. 72 y.o.; p=0.04). Complete blood counts were not different between the 2 groups. Low-risk MDS harbored significantly more abnormal karyotypes (27.8% vs. 4.2%; p=0.01) and higher numbers of mutations (1 vs. 0; p=0.04) compared to ICUS. Overall, most frequent mutations were TET2(14.6%), SF3B1 (12.2%) and ASXL1 (9.7%). Without mutation, the outcomes of low-risk MDS vs. ICUS were not different (p=0.53). Notably, low-risk MDS with any gene mutations showed inferior progression free survival at 5-year compared to the others (75.0% vs 96.4%; p=0.01). In multivariate analysis, IDH2 and DNMT3A mutations in low-risk MDS were associated with a
higher risk of leukemic transformation (HR=47.95 (4.5-510.5; 95% CI); p=0.001, HR=6.06 (1.3-29.4; 95% CI); p=0.03).

**Conclusions:** Mutation detection by NGS is important for proper risk stratification of patients with cytopenia.
Background And Aims: INTRODUCTION Recent studies have shown that OM-CMML and CMML have a similar clinical and biological profile. Although a high percentage of OM-CMML evolve to CMML, some of them die before evolving. Identifying predictive factors of evolution to CMML may be valuable since, as previously reported, OM-CMML that evolve to CMML show shorter overall survival (OS). AIM To identify factors for predicting OM-CMML evolution into CMML.

Methods: METHODS We analyzed clinical, genetic, and immunophenotypic data of a series of 94 patients diagnosed with OM-CMML (N=41) and CMML (N=53).

Results: RESULTS At a median follow-up of 42 months, 30% OM-CMML evolved to CMML. Patients with >3 mutated genes (HR:4.24, 95%CI 1.08-16.71, P=0.039) and monocytosis >20% (HR:3.48, 95%CI 1.05-11.47, P=0.041) showed a significant shorter time to CMML. These two variables were faced in a multivariate analysis and maintained their significance for predicting time to CMML (HR:4.33, 95%CI 1.23-15.20, P=0.022; and HR:5.82, 95%CI 1.32-25.7 , P=0.02). Moreover, these variables were also independent adverse prognostic factors for OS in our series of 94 patients (HR:4.39, 95%CI 1.99-9.68 , P<0.001; HR:3.05 , 95%CI 1.27-7.34 , P=0.013). Figure 1 depicts univariate survival analysis. Given the similar HR for predicting time to CMML of both variables, we implemented a model for predicting time to CMML: 0 points (none of them), 1 (one of them) or 2 points (both). This model offered an excellent
predictive power (C-index: 0.82).

Conclusions: CONCLUSION OM-CMML with higher molecular complexity and higher relative monocytosis are at greater risk of CMML evolution.
A MULTI-CENTER EXPERIENCE OF HYPOCELLULAR MYELODYSPLASTIC SYNDROMES (hMDS): FROM CLINICAL DESCRIPTION TO IMMUNOLOGICAL CHARACTERIZATION

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Background And Aims: Hypocellular Myelodysplastic Syndromes (hMDS) are a rare subset of MDS, defined by a reduced bone marrow (BM) cellularity, irrespective of WHO classification. We aimed to characterize the clinical features and outcome of hMDS patients and to identify common potential immune deregulations, which might account for hMDS-related BM failure and pathogenesis.

Methods: We compared overall survival (OS) of 336 hMDS and 1609 normo/hypercellular MDS (nMDS) enrolled in the national registry of the Italian Foundation of MDS (FISiM). BM and peripheral blood (PB) of twelve hMDS patients, recruited within FISiM NK-hMDS protocol, were also characterized by immunophenotypic and molecular analyses.

Results: According to R-IPSS groups, lower-risk hMDS had a median OS of 125 months (m) versus 74m of nMDS (p<0.001). Conversely, higher-risk hMDS showed a median OS of 19m, similar to 20m of nMDS. A clonal CD3+/CD8+/CD57+ T cell expansion was observed in 6/12 (50%) h-MDS patients, with 5/6 (83%) belonging to the Higher Risk group. Two of them also harbored a STAT3 activating mutation. On the contrary, a clonal CD3-/CD16bright/CD56bright/neg/CD57+/NKG2C+ NK cell expansion was found in 4/12 (33%) cases, with 3/4 (75%) included in the Lower Risk group.

Conclusions: Our data show an advantage in OS in Lower Risk h-MDS, compared to n-MDS. We also reveal peculiar immune alterations, which might represent novel prognostic biomarkers and relevant predictors of response to therapy. Further studies in a large series of patients are warranted to elucidate the actual meaning of these alterations and their clinical implications.
INCORPORATION OF COHESIN MUTATIONAL DATA INTO CURRENT IPSS-R CLASSIFICATION REFINES THE PROGNOSTIC STRATIFICATION OF VERY LOW/LOW-RISK MYELODYSPLASTIC SYNDROMES

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Background And Aims: The clinical impact of cohesin mutations in the myelodysplastic syndromes (MDS) is still undetermined. Thus, the aim of the study was the molecular and clinical characterization of MDS patients harboring cohesin mutations.

Methods: 418 MDS patients were analyzed by targeted deep sequencing using a custom panel of 117 myeloid-related genes, including cohesin genes: STAG1; STAG2; SMC1A; SMC3; RAD21; CTCF.

Results: The sequencing study identified 52 mutations in cohesin genes in 48 patients (11.5%). Regarding the molecular landscape of patients with cohesin mutations: mutated patients presented a significantly higher number of mutations ($p<0.0001$) and displayed a specific mutational profile with a strong co-occurrence between cohesin and splicing mutations ($p<0.0001$) and trisomy 8 ($p<0.0001$). Regarding the clinical characterization, patients with cohesin mutations displayed a poor prognosis disease phenotype, characterized by several cytopenia in blood, a higher number of blasts in bone marrow ($p=0.0014$) and a higher rate of progression to sAML ($p<0.0001$). Regarding the influence on clinical outcome, cohesin-mutated patients showed a shorter time to sAML progression (LFS: 1.4 vs. 8.3 years, $p<0.0001$) and a shorter overall survival (OS: 3.1 vs. 5.2 years, $p=0.069$). Noteworthy, the adverse impact on LFS was observed mainly in very low/low/intermediate-risk IPSS-R patients (multivariate analysis: HR 2.4, 95% CI 1.2-5.0; $p=0.015$). Furthermore, we assessed the prognostic value of cohesin mutations on the IPSS-R stratification. Of note, the OS and LFS of very low/low-risk patients who harbored cohesin mutations were statistically
significant shorter than those without these mutations, but nearly identical to intermediate-risk patients.

Conclusions: The incorporation of cohesin mutational data into current IPSS-R classification improved the prognostic stratification of very low and low-risk MDS patients.
CONDITIONAL RELATIVE SURVIVAL IN MYELODYSPLASTIC SYNDROMES REVEALS A PERMANENT EXCESS MORTALITY RISK FOR LONG-TERM SURVIVORS: A POPULATION-BASED STUDY IN THE NETHERLANDS

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Background And Aims: Most patients with myelodysplastic syndromes (MDS) experience excess mortality compared to an age- and sex-matched group from the general population, especially within the first few years post-diagnosis. Up-to-date statistics on the survival expectations of MDS patients who survived several years post-diagnosis are lacking. Therefore, we assessed the conditional 5-year relative survival (CRS) among MDS patients with long-term follow-up.

Methods: We selected all adult (≥18 years) MDS patients diagnosed in the Netherlands between 2001-2018—with survival follow-up through 2020—from the nationwide Netherlands Cancer Registry. Five-year CRS for each additional year survived up to 10 years post-diagnosis was calculated for three age groups (18-64, 65-74, and ≥75 years). Up-to-date statistics of 5-year CRS were estimated with the hybrid approach for the follow-up interval 2014-2020 (Figure). Excess mortality is considered minimal when the 5-year CRS is ≥95%.

Results: A total of 12,590 adult MDS patients (median age, 75 years) were included, of whom 5,956 (47%) were alive at some point during the follow-up interval and contributed to the survival estimates. There was a prognostic effect of age on 5-year RS at diagnosis (Figure). This effect essentially persisted up to ten years post-diagnosis (Figure). Excess mortality decreased with each additional year survived across all age groups (Figure). Nevertheless, excess mortality remains substantial across all age groups studied.
Conclusions: Significant excess mortality persists in MDS patients, even for those who survived up to ten years post-diagnosis. The excess mortality might be inherently tied to the incurable nature of most MDS subtypes, comorbidities, or late treatment-related mortality.
IGF-1R ACTIVATION SECONDARY TO HAPLOINSUFFICIENCY OF MIR-143 AND MIR-145 IS A TARGETABLE DEPENDENCY OF DEL(5Q) MDS

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Background And Aims: Chromosomal alterations frequently occur in MDS, with interstitial deletion of chromosome 5q (del(5q)) being the most common. First-line therapy, lenalidomide, is effective in 60 to 80% of patients and eventual resistance is common. The commonly deleted region (CDR) of chromosome 5q harbors several genes, including noncoding miRNAs, the loss of which contribute to disease phenotypes. miR-143 and miR-145 are located in the del(5q) CDR, but precise understanding of their role in human hematopoiesis and in the pathogenesis of del(5q) MDS is still lacking. The objective of this study was to 1. Examine the effect of miR-143/145 haploinsufficiency on hematopoietic stem and progenitor cells (HSPCs) 2. Identify other vulnerabilities due to miR-143/145 haploinsufficiency to target in lenalidomide-resistant del(5q) MDS.

Methods: We modeled haploinsufficiency of miR-143/145 using miR decoys in human CD34+ cord blood cells in vitro and in vivo to examine the impact on HSPC function. Bioinformatic analyses were used to identify potential targets of miR143/145 which were validated by RNA pull-down assays.

Results: We showed that IGF-1R is a target of miR-143/145 and increased IGF-1R expression (due to miR-143/145 depletion) in human HSPCs, provides both a growth advantage and increased survival. We showed that genetic or pharmacologic inhibition of IGF-1R blocks the growth advantage, and decreases viability of cells.

Conclusions: Our findings suggest a novel role of miR-143 and miR-145 as regulators of the proliferation and survival of HSPCs, through targeting of the IGF-1R pathway. This work thus provides a potential new therapeutic avenue for primary and lenalidomide-resistant del(5q) MDS.
ROLE OF ALLOGENEIC STEM CELL TRANSPLANT IN MYELODYSPLASTIC SYNDROME: A STUDY FROM THE ITALIAN FISIM REGISTRY

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Background And Aims: Only few MDS patients(pts) are transplant-eligible and only some of them proceed to HSCT, the sole curative option in MDS. We aimed to assess the actual proportion of MDS undergoing HSCT, the impact of pre-HSCT therapies on outcome and the reasons preventing eligible pts from HSCT.

Methods: We included 1293MDS pts prospectively enrolled in the FISiM registry between 1994 and 2019. HSCT eligible pts(n=211) were selected with the consensus criteria by De Witte et al(Blood 2017).

Results: Median age of HSCT-eligible pts was 64, 46% were at higher IPSS-R risk and 39% at intermediate risk. Sixty-seven pts underwent HSCT (5% of the study population and 32% of HSCT-eligible pts), 42% after intensive chemotherapy (IC), 41% after azacitidine and 17% upfront. Median survival after HSCT was 45 months and was not affected by pre-HSCT treatment. HSCT improved survival only in pts at higher IPSS-R risk (median 33 vs 14 months,p <0.001). The main reasons preventing eligible pts from HSCT were procrastination of HSCT and failure of cytoreductive treatment. Some lower risk pts (n=21) were considered for HSCT at progression when they were no longer transplant-eligible or progressed to AML(n=16) failing induction treatment. MDS-EB (n=134) received a cytoreductive treatment as bridge to HSCT that failed in 60% of cases, and only 16% was rescued by II line therapy undergoing HSCT.

Conclusions: HSCT-eligible pts should be promptly referred to HSCT-Centers to find the best window for transplant in lower risk pts and optimize the bridge therapy to HSCT in higher risk ones, also considering HSCT upfront.
APR 246 PLUS AZACITIDINE (AZA) IN TP53 MUTATED (M) MDS AND AML. LONG TERM FOLLOW UP OF PHASE 2 STUDY BY THE GFM


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Background And Aims: Our team and Sallman et al recently published results of 2 phase 2 studies combining AZA and APR246, a TP53 “reconforming agent” in TP53 mut MDS/AML (Cluzeau et al; JCO 2021). We report here long term follow up of our study.

Methods: Int, high or very high IPSS-R TP53m MDS and AML untreated adult patients were eligible. Patients received APR-246 4500 mg IV /d (6 hour infusions) (days 1-4) followed by AZA 75 mg/m²/d (days 4-10) in 28 day cycles.

Results: 52 patients were enrolled between Sept 2018 and July 2019 in 7 GFM centers: median age was 73 years (range 44-87), M/F: 28/25; 34 MDS (including 74% very high IPSS-R) and 19 had AML; 87% complex monosomal karyotypes. Only two patients are still on treatment after 19 and 26 cycles. As of 1 May 2021, with a median follow up of 12.5 months, median OS was 12.1 months, and 12.1, 14.8 and 3.0 months in MDS, AML (20-30% blasts) and AML (>30% blasts), respectively. Median response duration was 16.6 months (5.1-30.3+) OS was higher in patients reaching MRD negativity (5% sensitivity): median 16.6 vs 4.1 months, p<0.0001. 4 were allo-transplanted (all were still alive after 21.8 to 30.3 months). 11 patients were still alive after 21.8 to 30.3 months.

Conclusions: Our results suggest an OS improvement in TP53 mutated MDS/AML patients treated by AZA + APR 246, mainly in patients obtaining deeper response and allografted.
Background And Aims: Background: Therapy-related myeloid neoplasms (t-MN) are associated with dismal outcomes in otherwise long-term cancer survivors and are considered to be a direct consequence of DNA damage induced in haematopoietic stem cells (HSC) by cytotoxic therapy (CT). However, it largely ignores CT-induced changes to bone marrow (BM)-microenvironment. Aims: (i) to compare BM-microenvironment in t-MN with other-MN and age-matched healthy controls, (ii) to decipher CT-induced changes from MN induced changes in BM-microenvironment.

Methods: BM-mesenchymal stromal cells (MSC) phenotype, function, and whole transcriptome was compared in four well-selected cohorts: (1) t-MN, whereby MN occurred in cancer survivors following CT exposure; (2) MN in cancer survivors without prior exposure to CT (multiple cancers-MN; MC-MN); (3) primary-MN (p-MN) without preceding independent cancer and/or exposure to CT and (4) age-matched healthy controls (HC).

Results: t-MN-MSC exhibited altered morphology, proliferation, DNA damage repair, differentiation capacity and senescence compared to HC and other-MN (Fig.1A-B). t-MN-MSC also display Senescence Associated Secretory Phenotype and high expression of CDKN1A, a critical cyclin dependent kinase inhibitor orchestrating cell cycle arrest. Aberrant t-MN-MSC were unable to support haematopoiesis (Fig.1C). Transcriptome analysis showed reduced expression of genes involved in DNA damage repair, cell cycle regulation and HSC-support pathways (Fig.1D). Analysis of sequential BM samples elucidated impaired BM-MSC proliferation and differentiation following CT, well before diagnosis of t-MN.
Conclusions: This is the first comprehensive study demonstrating aberrant BM-microenvironment and providing molecular insight in t-MN. Moreover, our findings suggest that CT-induces long-term irreversible damage to BM-microenvironment which potentially contributes to t-MN pathogenesis.
LEUKEMOGENESIS IN GATA2 HAPLOINSUFFICIENT MICE IS A SECONDARY EVENT AFTER BONE MARROW FAILURE

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Background And Aims: MDS in young individuals is often not a random event but occurs in association with genetic predisposition. GATA2, a transcription factor shaping the hematopoietic system, is mutated in 7% of cases with RCC and 15% of cases with MDS-EB. The penetrance of myeloid neoplasia is exceedingly high in affected individuals (80% risk at the age of 40 years). Despite this high risk of MDS and leukemia, the mechanisms underlying malignant transformation are only insufficiently studied.

Methods: Using mice with GATA2 haploinsufficient hematopoietic system, we developed two model systems that allow to study leukemogenesis.

Results: In the first model, ENU was applied to introduce random mutations. Gata2⁻/⁻ leukemias developed significantly faster than their wildtype (WT) counterparts, but acquired driver mutations were similar in both genotypes. Interestingly, Gata2⁻/⁻ but not WT leukemias were preceded by bone marrow hypocellularity. In the second mouse model, leukemias developed spontaneously after stem/progenitor cell transplantation into lethally irradiated WT hosts. In sum, 15% of recipient mice succumbed to leukemia. Yet, leukemias emerged exclusively in mice that had developed BM failure first (40% of recipients). In contrast, 60% of recipients remained healthy. Leukemia development was always associated with somatic mutations and/or chromosomal alterations indicating clonal evolution. Profound transcriptomic changes in stem/progenitor cells indicate that epigenetic alterations might contribute to the different outcomes in our mouse model.

Conclusions: GATA2 haploinsufficiency predisposes to bone marrow failure in mice, which paves the way for secondary leukemia. Somatic events contribute to the different outcomes observed in our mouse model and can serve as prognostic markers.
Background And Aims: Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative therapy for most patients with juvenile myelomonocytic leukemia (JMML). Disease recurrence remains the major cause of treatment failure.

Methods: Outcome analysis of relapsed patients with JMML in the European Working Group of Myelodysplastic Syndromes in Childhood.

Results: Relapse occurred in 137 of 434 JMML patients (32%) who received allogeneic HSCT. The 5-year overall survival (OS) of all relapsed patients was 30%. Therapy approach for relapse included donor leukocyte infusion (DLI), low-dose (e.g. azacitidine) or intensive chemotherapy, and/or second HSCT. DLI (n=28) led to remission in 4 relapsed patients, but 3 of them died of complications. Chemotherapy alone was not effective for relapsed JMML. Majority of patients who did not receive second HSCT died shortly after relapse (no HSCT n=61, 5 year-OS: 8%). Remaining 76 patients (55%) received second HSCT; transplant outcomes were analyzed in 68 patients with sufficient data. The 5-year-OS, disease free survival, cumulative incidence of relapse and non-relapse mortality after second HSCT were 40%, 36%,
41% and 23%, respectively (med. follow-up: 7.7 years). Patients of older age at second HSCT (> 3 years) and early relapse (< 180 days) after first HSCT tended to have worse outcomes, while mutational subtype, type of donor, change of donor for second HSCT and conditioning regimens did not affect outcomes.

**Conclusions:** Second HSCT can achieve durable remission in patients with relapsed JMML, if it is feasible. Further efforts to reduce relapses after first HSCT and development of novel therapies for relapse are necessary.
APPRAISAL OF VENDOR-SUPPLIED NGS FILTER FOR CLINICAL VARIANT DETECTION IN MDS AND MDS/MPN PATIENTS

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Background And Aims: Our clinical NGS experience (accompanying abstract & submitted paper) highlights challenges of using vendor-supplied filters with conservative pipelines. We aimed to appraise the proprietary "Oncomine" filter (OF; ThermoFisher) for clinical use.

Methods: Oncomine™ Myeloid (ThermoFisher) NGS panel for 74 patients with confirmed or suspected MDS or MDS/MPN, comparing variants detected with OF versus a manual workflow including assessment with population databases (gnomAD), COSMIC, and IGV.

Results: 54/74 (73%) patients had at least one variant, with 169 total variants detected by manual variant calling (mean = 2.6 variants/patient). 63/169 (37%) did not match calls made by OF overall, while 49/169 (29%) did not match OF when excluding those that fell below the 5% variant allele frequency (VAF) cut-off applied for clinical reporting. Genes with most variants missed by OF include TP53 (11%), TET2 (8%), RUNX1 (6%), DNMT3A (6%), and EZH2 (6%). Furthermore, we had serial samples for 8/74 patients, including one MDS patient with four serial sequencing results. Findings for this patient were especially informative. Three clinically-relevant variants were not captured by OF, including in PRPF8 (VAFs of 55% and later 26%), and two ETV6 variants (14% and 21%; and 43% and 29%); especially important given the prognostic significance of ETV6 variants in MDS patients. Varying VAFs between these samples are attributed to selective pressures of azacitidine treatment undergone by this patient (10 cycles completed).

Conclusions: While the conservative OF is meant to prevent over-calling of non-clinically relevant variants, our results suggest that laboratories may wish to consider complementary software for clinical variant calling.
COMPARISON OF CYTOGENETIC ABERRATIONS IN 1590 PATIENTS WITH THERAPY-RELATED MDS (T-MDS) AND 4738 PATIENTS FROM THE REVISED INTERNATIONAL PROGNOSTIC SCORING SYSTEM DATABASE WITH PRIMARY MDS (P-MDS)


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Background And Aims: We performed an analysis on the distribution of karyotypes in therapy-related versus primary-MDS.

Methods: 1590 patients with t-MDS and 4738 with p-MDS were included.

Results: Some, like -7, abnormalities(7q), +1,der(1;7), and t(11q23) appeared more frequently in t-MDS. The largest differences were found in highly complex karyotypes. Del(5q), -Y, and +8 as single aberrations were more frequent in p-MDS, as highly complex in t-MDS. Del(11q) and del(20q) did not differ between p- and t-MDS. Some abnormalities occurring regularly in p-MDS, like del(12p), i(17q), and +21 are rare in t-MDS. Regarding p-MDS, del(5q), +8, -Y, del(20q), and del(11q) are more frequent as single abnormalities. In t-MDS most aberrations, especially del(5q), are more frequent in complex karyotypes. Isolated -7 occurred frequently after AML or lymphoma, while -Y often occurred after prostate, and del(5q) after breast cancer. –Y and del(5q) were found preferentially after radio-, -7/abn(7q) after chemotherapy. +8, del(11q), and del(20q) showed no preferences regarding pretreatment. As many t-MDS patients were treated in MDS phase, while more p-MDS patients were not, we tried to evaluate the potential bias on the karyotypes observed. Regarding cytogenetic risk categories (IPSS-R), treated patients had a higher-risk score, but still t-MDS patients had higher-risk karyotypes than p-MDS (very poor-risk: 6% in untreated, 12% in treated p-MDS, 24% in untreated, 38% in treated t-MDS). Good or intermediate-risk karyotypes, like +8, del(20q), and -Y showed, if isolated, no differences between treated and untreated patients.

Conclusions: Our results show substantial differences between therapy-related and primary-MDS regarding cytogenetic risk-groups and the occurrence of specific aberration patterns.
PIGN SUPPRESSES CIN AND MDS LEUKEMIC TRANSFORMATION/PROGRESSION VIA REGULATION OF THE SPINDLE ASSEMBLY CHECKPOINT

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Background And Aims: The spindle assembly checkpoint complex (SAC) is responsible for proper chromosomal segregation during mitosis and chromosomal stability. Our published data showed that PIGN expression aberrations were linked with chromosomal instability (CIN). Thus, we investigated the mechanistic link between PIGN, CIN and the SAC.

Methods: CRISPR/Cas9 gene knockout and siRNA gene suppression, and overexpression constructs were used to study the impacts of PIGN gene expression. Cell cycle suppression and release experiments were conducted. Co-immunoprecipitation experiments and confocal analysis were conducted to study the interaction among PIGN and SAC protiens.

Results: CRISPR/Cas9 mediated knockout of PIGN in CD34+ mononuclear cells derived from a healthy individual resulted in the suppression of MAD1 and MAD2. A similar observation was made in HEK293 PIGN knockout cells. PIGN loss in the HEK293 cells resulted in MAD1, MAD2, and MPS1 suppression but led to BUBR1 upregulation. PIGN downregulation resulted in impaired mitotic checkpoint activation and consequently impacted mitotic exit. PIGN downregulation results in defective mitotic checkpoint signaling and mitotic exit with an accumulation of missegregation errors. Interestingly, ectopic overexpression of PIGN restored the MAD1 and MAD2 expression. PIGN physically interacts with and regulates the SAC via MAD1, MAD2, MPS1 and BUBR1 during mitotic cell cycle progression. The co-purification of PIGN with some of these mitotic checkpoint proteins showed the direct role that PIGN may play in the regulation of mitotic checkpoint signaling.

Conclusions: PIGN as a CIN suppressor may be crucial in the regulation of mitotic integrity via the SAC as part of maintaining genome stability.
MOLECULAR FEATURES A PROGNOSTIC VALUE OF RAS MUTATIONS IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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**Background And Aims:** RAS mutations are recurrent events in myelodysplastic syndromes (MDS). However, there is limited data on the molecular features of RAS mutations in patients with MDS and its prognostic value remains controversial.

**Methods:** We conducted the 112-gene targeted sequencing in 776 patients with newly diagnosed primary MDS to detect RAS mutations. The mutual exclusivity and co-occurrence in gene mutations and clonal architecture were explored. Moreover, the prognostic significance of RAS mutations in MDS were analyzed.

**Results:** RAS gene mutations were found in 52(6.7%) cases. Of these, 38(4.9%) with NRAS mutation, 18(2.3%) with KRAS mutation, and 4(0.5%) with both. All of the NRAS mutations and 65 percent of KRAS mutations were located in the codon 12,13 and 61. PTPN11, FLT3, U2AF1, RUNX1, WT1, ETV6 and NPM1 mutations were enriched in patients with RAS mutations (Q<0.05). Around 80 percent of RAS mutations represented subclonal lesions in patients who harbored at least two different mutations. Patients with RAS mutations had higher levels of white blood cell count, neutrophil absolute count and bone marrow blast percentage (P<0.05), but lower levels of platelet count (P=0.048). Median overall survival of patients with NRAS mutations was shorter than the others (P=0.011) while the significance was lost in multivariable model. Whereas PTPN11, which is a RAS pathway-related gene, was an independent poor prognostic factor.

**Conclusions:** RAS gene mutations always occurred in late stage in MDS and co-occurred with other signal transduction and transcription factor related gene mutations. PTPN11, a RAS pathway-related gene, is an independent poor prognostic factor in MDS patients.
Background And Aims: Pediatric myelodysplastic syndrome (MDS) is a rare disease with a risk of progression to acute myeloid leukemia. Its incidence may be underestimated due to diagnosis difficulties. In this sense, cytogenetic analysis remains one of the essential pillars for diagnosis and prognosis. Nowadays, the standard tool to predict prognosis is the Revised International Prognostic Scoring System (IPSS-R) which included a new cytogenetic score, but it was based on adult patients' studies. The aim of this study was to analyze the frequency of cytogenetic abnormalities in pediatric MDS and their risk stratification according to IPSS-R, focusing on leukemic evolution.

Methods: We studied 181 patients (1-18 years) between 2000-2020 with MDS. Chromosomal analysis was performed using G-banding and FISH. The IPSS-R was applied based on cytogenetic risk stratification.

Results: Cytogenetic abnormalities were observed in 54.6% of all cases. The most frequent abnormalities were: -7 (23.8%), del(11)(q23) (11.3%) and complex karyotypes (8%). The AML evolution was present in 26.5% (48/181). According to IPSS-R, 6.6% of patients were classified as very good prognosis, 54.6% as good prognosis, 21.5% as intermediate, 13.2% as poor, and 4% as very poor prognosis. In these groups, leukemic evolution was observed in 50% (6/12), 9% (9/99), 28% (11/39), 54% (13/24), and 85% (6/7), respectively.

Conclusions: The predictive value of the cytogenetic risk of the IPSS-R was not consistent in patients with del(11)(q23) and +8, as these patients had MDS evolution. Thus, our results indicate the need for further studies to better classify the cytogenetic risk prognosis in pediatric MDS.
E-Poster Viewing

P01 / #8

Topic: AS01-Diagnosis / AS01a-Cytomorphology

E-POSTER VIEWING

CLINICAL AND GENETIC CHARACTERISTICS OF AML-MRC PATIENTS WITH MORPHOLOGIC DETECTION OF MULTILINEAGE DYSPLASIA

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Background And Aims: The category of AML with myelodysplastic syndrome (MDS)–related changes (AML-MRC) is distinguished from AML not otherwise specified (AML-NOS) by presence of multilineage dysplasia (MLD), preexisting MDS or MDS/ myeloproliferative neoplasm (MPN), or MDS-related cytogenetic changes. This study is to investigate the clinical and genetic characteristics of AML-MRC patients with morphologic detection of MLD alone.

Methods: A total of 112 patients with newly diagnosed AML-MRC or AML-NOS were included in the retrospective study. After collection of medical history information and in-depth morphological analysis, patients were divided into three groups: AML with MLD alone (AML-MRC-1; n=24), AML with history of MDS or MDS/MPN or MDS-related cytogenetics (AML-MRC-2; n=35) and AML-NOS (n=53). The clinical and genetic characteristics of the three groups were compared.

Results: Gender, age and platelet count of patients did not differ significantly between the three groups (P>0.05). However, there were statistically significant differences in white blood cell count, hemoglobin level and marrow blasts percentage (P<0.05, AML-MRC-2<AML-MRC-1<AML-NOS). The detection rates of abnormal karyotypes in the AML-MRC-1, AML-MRC-2 and AML-NOS group were 29.2%, 74.3% and 26.4% respectively, which showed significant difference (P<0.05). Abnormal karyotypes in AML-MRC-1 and AML-MRC-2 group consisted largely of complex karyotypes and MDS-related cytogenetics, while no such abnormal karyotypes were found in AML-NOS group.

Conclusions: Patients with MLD as sole AML-MRC criterion present high risk of complex karyotypes and MDS-related cytogenetics more often. Therefore, morphological analysis is crucial to separating these patients from category of AML-NOS.
INCIDENCE AND RELEVANCE OF T-LARGE GRANULAR LYMPHOCYTES AND NK CELLS IMBALANCES IN BONE MARROW BLOOD IN MYELODYSPLASTIC SYNDROMES

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Background And Aims: While T-Large Granular Lymphocyte Leukemia (T-LGLL) might be associated to MDS, a concurrent Chronic Lymphoproliferative Disorder of NK cells (CLPD-NK) has never been reported, NK cells (NKc) being usually scantly present the MDS bone marrow (BM). We aimed to characterize putative T-LGL and NKc expansions to identify potential new MDS prognostic markers.

Methods: Immunophenotypic evaluation of BM of 122 MDS patients was assessed by flow-cytometry. T-LGL clonality was evaluated by TCR gene rearrangement and NKc restriction (used as a surrogate of clonality) by Killer Immunoglobulin-like Receptors (KIR) expression. The presence of STAT3/STAT5b mutations by Sanger sequencing or allele-specific PCR was also investigated.

Results: Clonal T-LGL expansions were found in 13% (8/61) of MDS patients and were associated with lymphocytosis (p<0.05). Among these, STAT3 mutations were found in 5% of cases whereas no STAT5b mutations were identified. A KIR-restricted NKc increase was identified in the 8% (5/61) of MDS patients, satisfying CLPD-NK criteria in one case. A memory phenotype (CD56Dim/CD16High/CD57+/CD62L-) turned out to be a hallmark of these NKc proliferations. Although clonal T-LGL or NKc expansions were equally represented in high or low risk MDS, according to IPSS and R-IPSS, the presence of restricted NKc correlated with excess of blasts (EB, p<0.05).

Conclusions: We found that restricted NK cell expansions might contribute to the early identification of MDS-EB1/2, suggesting a previously unrecognized role for NKc in MDS evolution.
ADVOCATING FOR NGS IN THE CLINICAL SETTING; VARIANTS IDENTIFIED THROUGH MOLECULAR PROFILING ARE CLINICALLY ACTIONABLE IN MANY PATIENTS WITH CCUS, MDS, AND OTHER MYELOID MALIGNANCIES

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Background And Aims: We aimed to highlight the importance of integrating next-generation sequencing (NGS) into care of patients with known or suspected myeloid cancers – especially CCUS/MDS – by demonstrating the clinical impact of identified variants.

Methods: Prospective NGS was performed using the Oncomine™ Myeloid (ThermoFisher Scientific) panel (OMP) for 187 samples from 168 unique patients presenting at our site with suspected or confirmed myeloid malignancies and other hematological conditions. We have developed an actionability classification system specialized for myeloid malignancies (including MDS), by elaborating on existing systems for classifying somatic variants in cancer.

Results: The main diagnostic categories of the cohort include MDS (40%), AML (25%), and MPN (15%). Variants were detected in 68% of samples. Of 299 detected variants, 48% facilitated/clarified diagnoses, 29% affected prognoses, and 25% had the potential to influence clinical management (overall actionability = 77%). While age and diagnosis were independently predictive of overall survival ($p<0.001$ and $p<0.01$, respectively), so was the presence of ≥1 variant compared to 0 variants ($p=0.03$). Of note, OMP was essential to identifying patients with pre-malignant clonal states likely contributing to cytopenias (i.e. CCUS). Among 71 patients with suspected (but subthreshold for) MDS or MPN, clonality was detected in 36 (51%), while 35 (49%) had no variants, prompting further investigation of potentially reversible cytopenias or cytosis.

Conclusions: Given the clinical actionability of detected variants in our cohort, we advocate for more widely accessible, funded, myeloid NGS testing in the clinical setting to support the diagnosis, prognostication, and management of patients with myeloid malignancies – especially MDS.
PRESENCE OF PD-L1 IN MEGAKARYOCYTES OF PATIENTS WITH MYELODYSPLASTIC SYNDROME

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Background And Aims: The pathogenesis in Myelodysplastic syndromes (MDS) is characterized by the evasion of the immune system mediated by the recipients of the checkpoint programmed death 1 (PD-1), programmed death ligand 1 (PD-L1) and antigen 4 associated with cytotoxic T lymphocyte (CTLA4). Blocking these receptors has been used as a therapeutic target in MDS. The aim of the study was to associate the expression of PD-L1 with clinical manifestations in patients with MDS.

Methods: This is study involving 22 patients, diagnosed with MDS, attended at the Walter Cantídio Hospital, Brazil, in the period of 2018 to 2020. The study was approved by the HUWC ethics committee. Sociodemographic and clinical data were obtained from medical records. The expression of PD-L1 proteins was evaluated by immunohistochemistry. Statistical analyzes using the GraphPadPrism program and significance was p<0.05.

Results: The mean age was 70 years, with a frequency of (53.17%) in females, (93.54%) of the patients were considered to be very good or good cariotype. (41.94%) of patients were classified as MDS-DML and (70.96%) were considered very poor or poor prognosis. The expression of PD-L1 was observed in (68.18%) of the patients with scoring that ranged from weak, moderate and strong in bone marrow cells, while (31.82%) did not show scoring. Positive expression of megakaryocytes in PD-L1 was observed in (31.81%) patients. There was no significant association between PD-L1 expressions with sex, age, karyotype, classification and prognosis (p> 0.05).

Conclusions: In the present study, we observed intense PDL1 marking in megakaryocytes. Therefore, further studies are needed in order to assess the profile of PD-L1 proteins in MDS.
MDS WITH ISOLATED TRISOMY 8 AND DEL(20Q) – REALLY NOT MDS DEFINING?

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Background And Aims: In the WHO 2016 classification distinct cytogenetic abnormalities are considered definitive evidence of MDS even without significant morphologic dysplasia. However, the three rather frequent aberrations +8, del(20q) and Y-loss are not considered MDS-defining when morphologic criteria are not fulfilled. Therefore, we aimed to identify MDS-associated molecular mutations in patients with isolated +8 and del(20q) to find out whether the combination of cytogenetics and NGS might help to set the diagnosis MDS in cases with those cytogenetic abnormalities and undefined morphology.

Methods: 26 patients with isolated +8 (n=20) or del(20q) (n=6) were analyzed with NGS between 06/2019 and 04/2021. Cytogenetics were analyzed by banding and FISH. 19 of those patients were diagnosed with MDS, AML or CMML, 7 with MPN.

Results: We found 70 mutations associated with myeloid neoplasia in 24/26 patients (92%). Two of them did not show any mutations, both with isolated del(20q). All other patients had at least one mutation. The most frequently mutated genes were ASXL1 (n=9), SRSF2 (n=9), RUNX1 (n=7), NRAS (n=6).

Conclusions: For patients with suspected MDS but unclear morphology, cytogenetics is an important diagnostic tool. In cases with isolated +8 or del(20q) a diagnosis of myelodysplasia can be supported by molecular genetics. Most patients with those cytogenetic changes show at least one mutation strongly associated with myeloid neoplasia. Thus, the strict exclusion of trisomy 8 and del(20q) from definition of MDS is questionable. Further cases with morphologically uncertain MDS and isolated +8 or del(20q) will be included from the German MDS-Registry.
IMMUNOHISTOCHEMICAL AND CYTOGENETIC CORRELATION WITH TP53 MUTATIONS IN ACUTE MYELOID LEUKEMIA (AML) AND MYELODYSPLASTIC SYNDROME (MDS)

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Background And Aims: TP53 aberrations occur frequently in AML and MDS and are associated with adverse outcomes. Next-generation sequencing (NGS) for TP53 mutations, cytogenetic characterization of TP53 deletion, and immunohistochemical (IHC) staining for p53 are utilized clinically in decision making. Here, we aim to investigate the correlation of TP53 mutations with p53 expression and cytogenetic abnormalities in AML and MDS.

Methods: 32 cases (5 MDS, 27 AML) with TP53 mutations detected by NGS were subjected to fluorescence in situ hybridization (FISH) and IHC analysis. P53 staining with 2+ or 3+ intensity was defined as positive.

Results: A wide range of p53 staining patterns was detected (0-90%) (Figure), including 5 negative cases (15.6%), 9 (28.1%) with 1-10% staining, 10 (31.3%) showing 20-50% positivity, and 8 (25.0%) with >50% staining. 3 of the 5 negative cases had stop codon mutations, whereas majority of the positive cases (25/27) showed missense mutations. Among these, mutations involving the DNA-binding residue R273 (R273H and R273C) showed the highest frequency (5/27). Next, cytogenetic analysis revealed that 18/32 (56.3%) cases with TP53 mutations also harbor TP53 defect on the second allele, including 17p13 deletion (14 cases) or monosomy 17 (4 cases). Lastly, follow-up on a subset of patients revealed a high mortality rate (16/19, 84.2%) with most deceased within a year.

Figure. Representative images of p53 IHC staining
Conclusions: *TP53* mutated AML and MDS cases demonstrated a wide range of p53 immunoprofile and frequently co-occur with cytogenetic deletion. Integrating different measures of *TP53* status will provide a more complete picture to guide patient care.
EPIDEMIOLOGY OF OSTEOPOROSIS IN MYELODYSPLASTIC SYNDROMES: THE MYEOS PROSPECTIVE COHORT

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Background And Aims: In vitro and murine models studies supports the hypothesis of bone cells and hematopoietic stem cells interactions (including Wnt-B Catenin metabolic pathway and the FGF-23 hormone) in both physiological and pathological situations. A 2018 German social data study found an epidemiological association between myelodysplastic syndromes and osteoporosis. Our aim is to study the prevalence of osteoporosis in newly diagnosed MDS and to describe their bone and hematologic characteristics.

Methods: We started a prospective cohort based study of newly diagnosed MDS patients in the CHU of Angers, France. The patients then underwent a rheumatologic examination, bone densitometry, spine radiographs and bioology. An osteoporosis diagnosis was assessed if the bone densitometry T-scores were below -2.5 in one of the measured locations or/and one or more osteoporotic fracture antecedent. If needed, the patients received a bisphosphonate treatment.

Results: The first 54 enrolled patients of our cohort, including 23 women and 31 men, had a mean age of 76 years. 10 patients had abnormal karyotype, (4 very good prognosis, 4 intermediate karyotype, 1 poor and 1 complex karyotype). According to the R-IPSS classification, 13 (24,1%) patients were very low risk, 21 (38.9%) low, 15 intermediate (27.8%) 4 high risk (7.4%) and 1 (1.9%) very high . We diagnosed and treated 24 (44.4%) patients for osteoporosis. 3 patients had a hip osteoporotic fracture and 11 (20.3%) had one or more osteoporotic vertebral fracture.

Conclusions: Almost one in two newly diagnosed MDS patient were diagnosed with osteoporosis. A quarter of all included patients already had a main osteoporotic fracture (vertebral or hip).
TEN YEARS OF THE CZECH MDS REGISTRY: REAL LIFE DATA AND EXPERIENCES

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Background And Aims: We present data from 10 years of the Czech MDS group’s registry (MYDYS). Data was submitted by 9 university and 13 local centers. To date, 1,811 patients have been registered, with a yearly increase of 150-200 patients. We focused on patients treated with azacitidine and lenalidomide as the first groundbreaking options available when the register started.

Methods: Registry analysis

Results: Due to incomplete data, we finally analyzed 1610 patients. Median age was 70 (range 19-91), with 56.1% men, and maximum incidence between 70-74: 23/100,000. At analysis, 52% of patients were deceased. The main causes of death were progression (34.6%), AML transformation (14.5%), comorbidity (39.1%), infection (17.7%). We used the WHO 2016 classification, with leading diagnoses of MDS-MLD (27.3%) and EB II (24.3%). Complete cytogenetic results for IPSS-R were available for 1368 patients (68% good and very good; 13.7% intermediate; 18.3% poor and very poor). Major treatments were transfusions (40%), erythropoietin (20%) and azacitidine (35.5%). Only 6% of patients were transplanted. Median OS (in months) was highest in 5q- low risk patients (105.1), then MDS-RS (MLD and SLD; 58.2), MDS-MLD (49.4), CMML (24.7), EB I (23.2), EB II (15.6). Median OS was strongly influenced by performance status. IPSS and IPSS-R OS will be presented. Median OS on azacitidine treatment was 14.6 months. We also analyzed OS dependency on cytogenetic group, blast count and best response achieved with azacitidine and lenalidomide.

Conclusions: Our data reflects real life data from large registries worldwide. Our next goal is to implement NGS data and include new treatment modalities.
THE IMPACT OF GAINING OR LOSING TRANSFUSION INDEPENDENCE ON QUALITY OF LIFE IN MYELODYSPLASTIC SYNDROMES


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Background And Aims: Improving quality of life (QOL) has not been consistently observed in all MDS trials that demonstrate increased rates of transfusion independence (TI). Our objective was to longitudinally study and identify the key predictors of QOL and evaluate the impact of transfusion status changes on QOL.

Methods: We studied patients with MDS prospectively enrolled in the Canadian national registry who completed QOL questionnaires (EQ-5D, EORTC QLQ-C30 and Global Fatigue Scale) and underwent frailty, comorbidity and disability testing.

Results: A total of 891 patients were enrolled with a mean age of 73 years and a median follow-up of 19 months. They completed a median of 3 QOLs (range 1-16) every 6 months. While QOL scores tended to improve in the 65/268 (24%) of TD patients who became TI, only global QOL (QLQ-C30) significantly improved. In contrast, QOL scores declined significantly in the 143/623 (23%) of TI patients who became TD (figure 1). Global QOL as measured by the EQ-5D declined significantly over time in all patients (figure 2). Using multivariable analysis (general linear model) time, time varying transfusion status, Rockwood frailty, Charlson comorbidity score ≥3, any baseline disability and the use of azacitidine and or lenalidomide at any time were the covariates that provided the best model fit for EQ-5D global
Conclusions: These data suggest that QOL for patients with MDS diminishes over time in a predictable matter, with a course impacted by patient-related factors and the development of transfusion dependence.
Background And Aims: Hypomethylating agents (HMAs) are guideline-recommended treatments for patients with higher-risk myelodysplastic syndromes (HR-MDS); however, previous analyses have shown underutilization of HMAs in clinical practice. To better understand the impact of HMA non-use on resource utilization, this study compared the rate of hospitalizations and emergency room (ER) visits among HR-MDS patients according to use or non-use of HMA therapy.

Methods: This retrospective cohort study included patients from the 2010-2016 SEER-Medicare linked database who were diagnosed with refractory anemia with excess blasts (RAEB; a surrogate for HR-MDS) between 2011-2015, were aged ≥66 years at diagnosis, and had continuous enrollment ≥12 months prior to initial MDS diagnosis until death or end of study. Multivariable zero-inflated negative binomial models were used to assess the relationship between HMA use and number of hospitalizations and ER visits.

Results: Among HR-MDS patients (N=1,190), 664 (55.8%) were HMA users and 526 (44.2%) non-users. Mean (SD) age of patients at MDS diagnosis was 79.4 (6.9) years and 60.1% were male. HMA non-users had more hospitalizations (mean 0.47 vs 0.30, p<0.001) and ER visits (mean 0.69 vs 0.41, p=0.005) per month than HMA users. In multivariable analysis, the rate of hospitalization per month was 27% lower (incidence rate ratio [IRR]: 0.728, 95% CI: 0.648-0.819, p<0.0001), and the rate of ER visits was 32% lower (IRR: 0.684, 95% CI: 0.605-0.774, p<0.0001), for HMA users than non-users.

Conclusions: Non-use of HMAs among HR-MDS patients was associated with more hospitalizations and ER visits. Reasons for the underutilization of HMA therapy in clinical practice require further exploration.
ECONOMIC IMPACT OF TRANSFORMATION TO ACUTE MYELOID LEUKEMIA (AML) AMONG PATIENTS WITH HIGHER-RISK MYELODYSPLASTIC SYNDROMES (HIGHER-RISK MDS) IN THE UNITED STATES: RETROSPECTIVE MATCHED COHORT STUDY

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Background And Aims: Patients with higher-risk MDS who transform to AML may experience rapid disease progression, leading to higher healthcare utilization and costs. To describe this economic impact, we compared hospitalizations and costs between patients who experienced transformation to AML and those who did not.

Methods: Patients with higher-risk MDS (≥18 years old) initiating first-line therapy during 1/1/2008–6/30/2019 were identified in the Optum database. Patients who transformed to AML were matched 1:1 to patients who did not, based on follow-up duration. Three study periods were defined: entire follow-up; pre-AML (before transformation); and post-AML (after transformation). For patients who did not transform, pre- and post-AML periods were determined using the transformation date of their matched pair. Hospitalizations and costs (2019 US$) were compared after adjusting for baseline differences.

Results: A total of 118 matched patient pairs were included and followed for a median of 12 months. During the pre-AML period, there were no differences in hospitalization rate or costs between patients who transformed to AML and those who did not. The hospitalization rate was significantly higher in patients who transformed than those who did not during the entire follow-up (58.5% vs 44.1%; p=0.0295) and post-AML (47.5% vs 28.0%; p=0.0028) periods. Adjusted mean monthly total costs per patient were significantly higher for patients who transformed than those who did not during the entire follow-up (+∆$6,815; p<0.0001) and post-AML (+∆$21,564; p<0.0001; Figure)
Conclusions: AML transformation significantly increased hospitalizations and costs over ~12 months, highlighting the importance of treatments that delay transformation.
LIVING WITH MPN FATIGUE

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**Background And Aims:** Myeloproliferative neoplasms (MPNs) are rare haematological cancers. Studies report fatigue as the most common MPN symptom impacting quality of life. There is limited research into how fatigue affects the lives of people with MPN. This study aimed to gain insight into the lived experience of fatigue in MPN.

**Methods:** People diagnosed with MPN were invited to complete an online survey and if eligible, express interest in further participation. Semi-structured interviews and focus groups explored participant’s experience of fatigue. Thematic analysis was used to develop themes describing the lived experience of fatigue.

**Results:** Twenty-three people with an MPN participated in interviews and focus groups. Results show how fatigue affects the functional, social/family and emotional wellbeing of participants. Four themes describing the experience of fatigue in MPN were developed. (1) Life with an MPN explains the lived experience of the MPN diagnosis. (2) “It’s not being tired, it’s completely different. It’s fatigue” relates how fatigue feels. (3) “It changes your life completely” describes fatigue’s impact on daily life. (4) “Living the best life I can” involves professional advice and self-help approaches tried by participants. These findings highlight the multifactorial nature of fatigue and the absence of information surrounding the experience of it.

**Conclusions:** Fatigue in MPN can affect all aspects of health, wellbeing and general life, yet is seldom addressed by health professionals. This raises issues of awareness and capacity to respond. A greater understanding of fatigue as a symptom of MPN is urgently needed to help improve patient quality of life.
TRANSFUSION BURDEN AMONG LOW-RISK MDS PATIENTS

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Background And Aims: A high proportion of patients with lower-risk MDS become dependent on red-cell transfusions, a situation associated with reduced quality of life and overall survival[1]. In 2020, a patient, physicians and nurses survey was carried out to identify the impact of blood transfusion (BT) on low risk MDS patients’ quality of life perception and daily life organization as well as the perception of transfusion burden by caregivers. This study was jointly conducted by the patient association Connaître et Combattre les Myélodysplasies (CCM) and Celgene (SAS). [1] Fenaux et al., N Engl J Med 2020;382:140-51.

Methods: A two steps survey: a qualitative phase with 10 MDS patients, followed by a quantitative phase with 40 hematologists, 10 nurses- in blood transfusion centers-, and 41 transfused MDS patients.

Results: On average, BTs were performed every 3 weeks. All interviewed patients used a single public BT public facility. BT sessions lasted from ½ to 1 day, and were perceived as tiring for 25% of patients. 73% of patients shared a room with other transfused patients. 27% received emergency transfusions. BT 18 days short terms effects (78%) and iron overload (60%) was the patients’ main concerns. Main constraints: • MDs: Logistics, going to the hospital •Nurses: BT frequency and schedule modifications • Patients: BT frequency and treatment of iron overload.

Conclusions: Potential axes of BT burden identified through the survey: • Decrease waiting time, and shorten BT time. • Reduce BT frequency to allow patient’s mobility. • Provide more information on BT. • Develop therapeutic alternatives to avoid BT.
QUALITY OF LIFE IS IMPAIRED IN ANEMIC MDS PATIENTS, ESPECIALLY WHEN HB DROPS BELOW 9

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Background And Aims: Quality of life (QoL) is impaired in MDS, as shown with EUMDS data. Aim: Study QoL for various levels of anemia in MDS patients.

Methods: Patients in The Israel MDS registry (EUMDS member) fill out the EQ-5D QoL questionnaire. The evaluated parameters are: mobility, self-care, daily activities, pain/discomfort and anxiety/depression, each scored 0(normal), 1(mild/moderate), or 2(poor). They also evaluate their general health status using a visual analogue scale (VAS), scoring from 0 (poor) to 100(excellent). Anemia was classified as none/normal (Hb≥12.5 g/dl), mild (10≤Hb<12.5), moderate (9≤Hb<10), severe (8≤Hb<9) or very severe (Hb<8).

Results: In total, 126 MDS patients participated: 19, 40, 17, 21 and 29, from normal to very severe anemia, respectively. Figure 1 shows mean QoL of the 5-parameters for all patients (A), and for each individual (B), as well as the mean VAS score for all patients (C) and for each individual (D). Anemic patients show a wide QoL variability (patients with the same Hb behave differently, Figure1, B, D). Also, note the drop in QoL from moderate to severe anemia (below 9 g/dl, p=0.06 for 5-parameter; p=0.01 for
Conclusions: In this preliminary study: 1) Poor QoL in anemia of MDS is variable and not linear, suggesting that other factors, in addition to Hb, affect QoL. 2) The sharp drop in QoL with Hb < 9 g/dl (Figure 1, A and C from blue to pink), might lead to a therapeutic paradigm shift, with transfusion recommendations for patients with Hb < 9. These findings must confirmed with larger patient cohorts.
CU1STOM IMAGE ANALYSIS PIPELINE FOR AUTOMATED IMMUNOHISTOCHEMISTRY QUANTIFICATION DEMONSTRATES A DNA DAMAGE PHENOTYPE IN TP53-MUTATED MYELODYSPLASTIC SYNDROME

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*Background And Aims:* A DNA damage phenotype in myelodysplastic syndrome (MDS) has been proposed previously based on flow cytometric evaluation of phospho-Histone H2A.X signal at sites of DNA double-strand breaks. In this study, we have developed an automated image analysis pipeline to identify MDS subgroup exhibiting a DNA damage phenotype.

*Methods:* Formalin-fixed and paraffin-embedded (FFPE) bone marrow core biopsies were sectioned at 2-micron thickness for immunohistochemistry (IHC) using a well-characterized pH2A.X antibody (clone JBW301). A training cohort of 36 MDS (SLD/MLD, RS, EB) or Acute Myeloid Leukemia cases were included with molecular annotations, along with 20 non-malignant cases as control. Automated nuclei detection on 1900 Hematoxylin-DAB images (at 100X) were performed using QuPath. Custom Matlab algorithms automated grading of nuclear pH2A.X staining (0, 1+, 2+, 3+), and the data management of 260,000 analyzed nuclei. Original and post-processed images were stitched side-by-side for manual inspection, and pH2A.X H-score exported to Prism for statistical analysis.

*Results:* Punctate to diffuse nuclear pH2A.X staining is often seen in erythroid clusters, myeloid progenitors, and occasionally in neutrophils. Megakaryocytes and megakaryoblasts also show staining in some cases. TP53-mutated MDS/AML shows significantly higher pH2A.X H-score as compared to control (p < 0.01; Dunn’s multiple comparisons test).

*Conclusions:* We created a custom image analysis pipeline for automated IHC quantification, and demonstrated higher pH2A.X staining in TP53 mutated MDS/AML. We will use this tool to further study the interaction between marrow microenvironment and DNA-damage, as well as to perform other phenotypic characterization to help elucidate the functional underpinning of major MDS molecular subgroups.
INDUCTION CHEMOTHERAPY AND ECULIZUMAB TREATMENT OF HIGH-RISK MYELODYSPLASIA WITH A LARGE PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA CLONE

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Background And Aims: Paroxysmal nocturnal haemoglobinuria (PNH) is a rare clonal hematologic disorder associated with myelodysplastic syndromes (MDS). We present an unusual case of a large PNH clone complicating MDS with complex genetics to illustrate the simultaneous treatment of both conditions.

Methods: Morphological, flow cytometric, cytogenetic and molecular genetic analysis were performed in-house using standard methods. PNH clone quantification was performed using multiparameter/FLAER flow cytometry.

Results: A 62 year old woman was admitted with a two month history of fatigue and was found to be pancytopenic. Bone marrow biopsy demonstrated IPSS-R very high risk MDS, with a complex monosomal karyotype and TP53 mutations. Peripheral flow cytometry demonstrated the presence of a large PNH clone (Table 1). Weekly eculizumab treatment alongside induction chemotherapy (daunorubicin/cytarabine/gemtuzumab ozogamicin) was given. After one cycle morphological and flow cytometric remission was achieved and PNH clone size fell to <3% (Fig. 1), however genetic aberrations persisted. Of particular note, no thrombotic or haemorrhagic complications occurred during treatment.
<table>
<thead>
<tr>
<th><strong>Modality</strong></th>
<th><strong>Result</strong></th>
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<tbody>
<tr>
<td>Karyotype</td>
<td>42,XX,add(2)(p11.1),del(5)(q13q33),del(6)(q13q25),-7,-10,-10,add(13)(q34),-16,add(17)(p11.2),-18,add(20)(q11.1),+mar</td>
</tr>
<tr>
<td>Myeloid gene panel</td>
<td>TP53c.396G&gt;T p.(Lys132Asn)[VAF 6%] TP53c.783-1G&gt;A p.? (VAF)[VAF 56%]</td>
</tr>
<tr>
<td>BM flow cytometry</td>
<td>13.5% myeloid progenitors: 34+/117++/13+/33+/DR+/MPOwk+ 15% neutrophils: CD56-/DR-/abnormal 1 27% monocytes: DR low, absent CD14</td>
</tr>
<tr>
<td>Peripheral PNH screen</td>
<td>FLAER-CD14neg monocytes: 96.6% FLAER-CD24neg granulocytes: 92.3% CD59neg erythrocytes: 0.3% CD-59neg reticulocytes: 22.7% Type-III-CD-59neg reticulocytes: 56.6%</td>
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**Conclusions:** A large PNH clone is described alongside MDS with excess blasts and a complex karyotype with TP53 mutations. This is the first description of combination treatment with induction chemotherapy and eculizumab. Our patient achieved an MRD positive remission after one cycle with no major adverse events.
MUTATIONAL PROFILING OF CLONAL HEMATOPOIESIS, MDS AND SAML DEPICTS DIVERSITIES OF CLONAL PROGRESSION

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Background And Aims: Clonal hematopoiesis (CH) is defined by the presence of genetic alterations in the peripheral blood (PB) of individuals without clinical manifestation of a hematologic malignancy. However, CH is associated with an increased risk for progression to myelodysplastic syndromes (MDS) or acute myeloid leukemia (AML). We genetically characterized CH, MDS and secondary AML to depict the changes of the mutational spectrum associated with progressing clonal alterations of the hematopoietic system.

Methods: Samples from individuals without myeloid neoplasia undergoing hip replacement surgery (n=261), patients with MDS (n=92) or sAML (n=123) were screened for variants in 68 leukemia-associated genes using a targeted sequencing approach (variant allele frequency [VAF] cut off, 1%). Follow-up (FU) PB samples were available for 21 individuals with CH and 16 untreated low-risk MDS patients, 6-24 months after screening.

Results: At screening, we detected variants in 127/261 (49%) healthy individuals, 84/92 (91%) MDS and 117/123 (95%) sAML samples, with median VAFs of 2.7%, 18.8% and 37.1%, respectively. CH, MDS and sAML showed entity-specific mutation profiles. Most variants in CH affected epigenetic modifiers, while mutations in splicing factors, signaling pathways and transcription factors increased with clonal progression (see Figure 1). During FU, untreated low-risk MDS patients more frequently gained additional mutations compared to CH individuals (7/16 vs 2/21, respectively; p=0.024). However, we did not observe significant changes in clone sizes over
Conclusions: CH, MDS and sAML show characteristic mutation profiles that remain relatively stable over a 6–24-month period. Gains of additional variants and clonal expansion associate with disease progression.
DIFFERENTIAL CLINICAL AND MUTATIONAL PROFILE OF HYPOPLASTIC MYELODYSPLASTIC SYNDROMES

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Background And Aims: According to 2017 WHO Classification, most myelodysplastic syndromes (MDS) have normal or increased bone marrow cellularity (NH-MDS), but 10-20% have hypocellularity (h-MDS)(Yao, et al. Oncotarget. 2016). The applicability of the IPSS-R is debated and there are controversy regarding the mutational profile of h-SMD (Nazka, et al. Haematologica 2015). Aims: to investigate the mutational risk profile of h-MDS and to correlate with the survival advantage described by some authors for h-MDS.

Methods: Prospective study of MDS, categorized as NH-MDS, del(5q) and h-MDS. We analyzed by NGS the number of driver mutations (DM), High Risk mutations (HRM): TP53, RUNX1, ASXL1, EZH2, ETV6, U2AF1 (Bejar, et al. Blood 2015), score DM, score HRM, VAF.

Results: 163 MDS, age 66 (73 W/90 M). 43 h-MDS, 109 NH-MDS and 11 del (5q), no differences in MDS subtypes between the 3 groups (P 0.714). Lower age, blast, cytogenetic risk in h-MDS (62 vs. 72) (P 0.001), (2 vs. 3) (P 0.001) and Intermediate vs. Poor(P 0.034), without differences in IPSS-R (P 0.225). Number of DM 370, higher in NH-SMD vs. h-SMD (91.5% vs. 50%; P 0.001), score DM (3.0 vs 2.0; P 0.022). 110 patients HRM, higher in NH-MDS (33.3% vs 16.4%) (P 0.011). Lower mutations on splicing (9.8% vs. 31%), SF3B1 (1.6% vs. 7.1%) (P 0.045) and TP53 (0% vs. 9.9%; P 0.010) in h-MDS. Higher 5-year OS in SMD-h (92.8% vs 52%)(log-rank, P 0.025)

Conclusions: More favorable profile, lower cytogenetic and mutational risk, less frequent mutations, HRM, TP53, and splicing machinery and superior 5-year survival in h-MDS
SECOND GENERATION SEQUENCED GENE MUTATION CHARACTERISTICS OF MDS PATIENTS WITH NORMAL CHROMOSOMES

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Background And Aims: Myelodysplastic syndrome (MDS) development is a dynamic process via clonal origination, competition and dominance. The aim of the present work is to analyze the gene mutation characteristics for MDS patients with normal chromosomes.

Methods: A total of 39 genes were chosen as an assembly to perform the second generation sequencing for 352 MDS patients with normal chromosomes at point of diagnosis, compared with 268 cases that had abnormal chromosomes. Then 16 of the 352 cases underwent repeat sequencing under the premise that the AML (acute myeloid leukemia) transformation did not occur. In addition, 22 MDS/AML patients accepted paired analysis between the sequencing results immediately after AML transformation and the diagnostic point.

Results: The mutation frequency reached 81.2% for the 352 cases, with insignificant difference for the initial mutation (involving ASXL1/DNMT3A/TET2) when compared to controls. Rare special chromosome-related gene mutations were checked for this subset of patients, such as TP53, RUNX1 and U2AF1 for complex, chromosome 7 involvement, and 20q-/+8, respectively. Only epigenetic–related BCOR mutations were more common in this subset. Ten of 16 cases who underwent repeat sequencing showed mutation evolution, with or without disease advance. However, the evolution never implicated active signaling. Whereas, 18 paired samples of the 22 MDS/AML were defined with AML transformation-related mutations (active signaling, myeloid transcription, cancer suppressor).

Conclusions: In general, MDS with normal chromosome is the early stage. Sequencing should be helpful for disease diagnosis especially for those with lack of morphological features. Last hits are necessary for MDS to be transformed into MDS/AML.
INCREASED MORTALITY AMONG SMOKERS WITH MYELODYSPLASTIC SYNDROMES (MDS)

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¹Jacobi Medical Center, Albert Einstein College of Medicine, Internal Medicine, Bronx, United States of America, ²Montefiore Medical Center, Albert Einstein College of Medicine, Medical Oncology, Bronx, United States of America

Background And Aims: Smoking has been shown to be an independent risk factor for MDS. We aimed to assess whether smoking is associated with worse outcomes among patients with MDS at Montefiore Medical Center, Bronx, NY.

Methods: Patients with MDS and CMML diagnosed between June, 2000 and November, 2020 were analyzed. Those without available tissue diagnosis or smoking history data were excluded. Descriptive statistics compared ever-smokers to non-smokers. Cox PH regression was used to analyze the risk of transformation to AML and mortality among the 2 groups and multivariable analysis (MVA) adjusted for age, sex, de novo disease and R-IPSS.

Results: Out of the 147 patients identified, 109 (74.1%) had a diagnosis of de novo MDS and 89 (60.5%) had history of active or former smoking. Smokers were predominantly males (66.3%) vs non-smokers (37.9%) (p=0.001). Smokers were diagnosed more frequently with high or very high risk MDS, although the difference was not statistically significant (38.1% vs 28.6%, respectively; p=0.28). TP53 mutations were numerically more frequent among smokers (24.4%) compared to non-smokers (12.8%) (p=0.16). Median follow-up time for smokers and non-smokers was 19.4 and 31.4 months, respectively. In MVA, there was a trend for increased risk of AML transformation in smokers vs non-smokers (HR 2.03, 95% CI 0.99 – 4.15; p=0.052). Smokers with MDS had significantly greater mortality compared to non-smokers (HR 2.08, 95% CI, 1.22 – 3.54; p=0.007).

Conclusions: Smoking was associated with worse survival among MDS patients in our cohort. Although not significantly different, the prevalence of TP53 mutations was higher among smokers. Larger studies are warranted.
SIMILAR MOLECULAR PATTERN IN T-AML IN PATIENTS TREATED WITH PARP INHIBITORS FOR METASTATIC OVARIAN CANCER

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Background And Aims: Recently, Morice published regarding the occurrence of AML and MDS in patients receiving Poly(adenosine diphosphate-ribose) polymerase (PARP) inhibitors therapy. They found that the incidence of MDS and AML across PARP inhibitor groups was 0·73% as compared to 0·47% across placebo groups.

Methods: In our experience we followed 450 patients affected by metastatic epithelial ovarian cancer (OC) BRCA+ treated with PARPi after chemotherapy and we found 8 consecutive patients who developed therapy-related AML (t-AML) in a short period between June 2019 and December 2020. Patients' characteristics were: Median age at OC diagnosis was 55y (range 45-70), the median number of chemotherapy lines before PARPi treatment was 3, the median interval between PARPi treatment and the onset of t-AML/MDS was 14 months.

Results: None of them had recurrent molecular alterations including NPM1 mutations or FLT3-ITD, but when we analyzed subclonal mutations by an NGS approach we found that in all of them TP53 mutations were recurrent. We found the following TP53 mutations: c.376–1G>A (splice acceptor), c.78delT and c.524G>A. The median VAF was 25%. Moreover in two of them we were able to study the ovarian tissue collected at cancer diagnosis and the TP53 mutations were not present. In 2 out 8 patient we found also DNMT3A mutations. In our series the incidence of t-AML/MDS was 1.7%.

Conclusions: We confirm that there is a relation between the use of PARPi and the development of s-AML, but we believe that the incidence of secondary hematological malignancies could be underestimated in this setting.
THE DEL(5q) SIZE IN MDS IS CORRELATED TO KARYOTYPE COMPLEXITY AND FREQUENCY OF TP53 MUTATIONS.

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Background And Aims: The most common cytogenetic abnormality in MDS is del(5q) occurring either as a sole aberration or as a part of complex karyotypes (CK). Isolated del(5q) is associated with favorable outcome while MDS with CK relate to poor prognosis. It remains unclear whether deletion size matters for different MDS phenotypes. The aim was to compare the extent of del(5q) in MDS and to assess its relationship with TP53 mutation.

Methods: Extent of del(5q) was analyzed using mBAND (MetaSystems) and aCGH/SNP (Illumina, Agilent). Sequence analysis of TP53 gene was performed using NGS on a 454 GS Junior system (Roche) or MiSeq sequencing instruments (Illumina).

Results: In the group of 175 cases with isolated del(5q), the most frequently deleted segment ranged between 5q14-5q33.3, with the smallest deletion encompassing the 5q31.1-5q31.3 region (18.527Mb). TP53 mutations were proved in 19.4% cases. In the group of 173 cases with CK, deletion often involved entire long arm including telomeric region. Mutations of TP53 and/or LOH17p were detected in 49% of them. The CDRs were located at 5q31.1 (5.522Mb) in cases with isolated del(5q) and between the bands 5q31.1-5q31.3 (18.527Mb) in cases with CK.

Conclusions: Patients with isolated del(5q) had a smaller size of deleted segment. More extensive 5q deletion was associated with higher karyotype complexity, increased frequency of TP53 aberrations and worse prognosis. Accurate analysis of breakpoints and del(5q) size points out to the correlation of deletion size with increasing genomic instability in MDS and contributes to a better understanding of the MDS pathogenesis. RVO-VFN64165, MHCR 00023736
Background And Aims: To explore the clinical implications and prognostic value of TP53 gene mutation and deletion in patients with myelodysplastic syndromes (MDS).

Methods: 112-gene targeted sequencing and interphase fluorescence in situ hybridization (FISH) were used to detect TP53 mutation and deletion in 584 patients with newly diagnosed primary MDS. The association of TP53 mutation and deletion with clinical features and their prognostic significance were analyzed.

Results: Alterations in TP53 were found in 42 (7.2%) cases. Of these, 31 (5.3%) cases showed TP53 mutation only, 8 (1.4%) cases showed TP53 deletion only, 3 (0.5%) cases harbored both mutation and deletion. A total of 37 mutations were detected in 34 patients, most of them (94.6%) were located in the DNA binding domain (exon 5-8), the remaining 2 were located in exon 10 and splice site respectively. Patients with TP53 alterations harbored significantly more mutations than the others (P=0.016). The median age of patients with TP53 alterations was higher than their counterparts (60 versus 52 years, P=0.029). TP53 alterations correlated with complex karyotype and higher-risk categories in International prognostic scoring system (P<0.001). Median overall survival of patients with TP53 alterations was shorter than the others (13 months versus not reached, P<0.001) while the significance was lost during complex karyotype adjusted analysis in multivariable model.

Conclusions: TP53 mutation was more common than deletion in MDS. The majority of mutations were located in the DNA binding domain. TP53 alterations were strongly associated with complex karyotype and always coexisted with other gene mutations. TP53 alteration was no longer an independent prognostic factor when patients occurred complex karyotype in MDS.
MUTATIONS IN RUNX1 GENE CONTRIBUTE TO THE PROGRESSION OF LOWER-RISK MDS PATIENTS BY DISRUPTING CELLULAR PROTECTION AGAINST MALIGNANT TRANSFORMATION


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Background And Aims: Despite the risk category, part of lower-risk MDS patients (LR-MDS) progresses rapidly. The identification of these patients and early intervention may improve their outcomes. Thus, this study aimed to determine molecular markers of rapid progression. Subsequently, we analyzed changes at the transcriptome level to reveal the mechanisms underlying the malignization.

Methods: DNA samples of 214 LR-MDS patients from the time of diagnosis were sequenced using TruSight Myeloid Sequencing Panel (Illumina). 19% of patients progressed within 5 years. RNA from CD34+ cells (8 LR-MDS patients with and 29 without RUNX1 mutation, 20 higher-risk MDS) was sequenced using NEBNext-Ultra-II-Directional-RNA-Library-Prep-Kit (New England Biolabs). Differentially expressed genes were analyzed by String 11.0 and GSEA.

Results: Pathogenic mutations were identified in 137 (64%) patients and the presence of mutations significantly affected PFS (p=0.0016). Out of all mutated genes, RUNX1 was the most significant variable. Its presence shortened the median of PFS from 65 to 25 months (p<0.0001). Integration of RUNX1 mutational status into IPSS-R distinctly separated RUNX1 patients from others. RNA-Seq revealed downregulated expression of DNA damage response, chromatin silencing and cellular senescence pathways in RUNX1 LR-MDS patients. Moreover, clustering of all MDS patients showed that gene expression profiles of RUNX1 LR-MDS resemble higher-risk MDS more than other LR-MDS.

Conclusions: In conclusion, sequencing of LR-MDS patients at the time of diagnosis could unveil patients at risk of progression and refine patient care. Mutations in RUNX1 gene possibly contribute to the rapid progression by deregulation of pathways protecting cells against malignant transformation. Funding: AZV NV18-03-0022, MH CZ-DRO (UHKT,00023736) and (VFN,64165).
NONCODING RNAs IN MDS PATHOGENESIS AND THEIR PREDICTIVE VALUE IN AZACITIDINE RESPONSE

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Background And Aims: Prediction of response to azacytidine (AZA) is an important challenge in MDS research. In addition to protein-coding genes (PCGs), AZA efficiency can be influenced by various noncoding RNAs (ncRNAs). Our aim was to provide biologic insights into contribution of ncRNAs to mechanisms of AZA treatment and propose novel markers for response prediction.

Methods: We performed RNA sequencing in CD34+ bone marrow cells of 26 patients with higher-risk MDS/AML with myelodysplasia-related changes before AZA therapy and 10 healthy controls. Along to PCGs, we examined transcription of long ncRNAs (lncRNAs), circular RNAs (circRNAs), and transposable elements (TEs).

Results: We compared four categories of transcripts (PCGs, lncRNAs, circRNAs, and TEs) and found that lncRNAs have the strongest potential to predict AZA response, even overperforming prediction power of PCGs, whereas circRNAs and TEs do not provide significant potential to identify AZA responders. Combined set of the best predictors finally included 19 genes. Interestingly, 14 of them were lncRNAs, whereas only four PCGs, one circRNA, and none TE were included in the set of predictors. Pathway analysis suggested epigenetic regulation and recombinational repair as crucial for MDS/AML cells with respect to AZA response, and network modelling defined three deregulated IncRNAs (CTC-482H14.5, RP11-419K12.2, and RP11-736I24.4) associated with these two processes.

Conclusions: We showed that AZA treatment can affect expression of various ncRNAs, substantially contributing to clinical effects of the drug, and defined new potential biomarkers able to predict AZA response. Supported by AZV CR (17-31398A and NU20-03-00412), GA CR (N20-19162S), and MH CZ-DRO (UHKT, 00023736).
THE COMBINATION OF A RAS SIGNALING MODULATOR WITH AZACITIDINE IMPROVES HEMATOPOIESIS IN VIVO AND HAS IN VITRO EFFECTS ON METABOLIC/DIFFERENTIATION PATHWAYS AND INNATE IMMUNE SIGNALING

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Background And Aims: Although Azacitidine (AZA) is the mainstay treatment for higher-risk MDS patients, almost all patients become resistant to treatment. The addition of a novel Ras mimetic that inhibits Ras/Raf signaling, Rigosertib (RIGO), yields a response rate of 54% of HMA failures and results in significant improvement in hematopoiesis overcoming the epigenetic clinical resistance phenotype but the mechanism is still elusive.

Methods: We investigated the protein expression in response to different treatment (AZA, RIGO and sequential combinations (SC) RIGO/AZA) in vitro in MDS-L cell line by protein-array and further performed functional and immunophenotyping assays.

Results: We previously demonstrated that RIGO/AZA upregulates RIG-I, Wnt/B-catenin and hematopoiesis signaling pathways. In this study we report downregulation of PIK3R1, AKT1, mTOR, p38 MAPK, PTEN, RPS6KA1 and upregulation of mitochondrial and oxidative phosphorylation related gene in MDS-L cells treated with RIGO/AZA. Additionally, we found that AZA increases the percentage of CD34+CD38+ cells, indicative of differentiation, whereas RIGO alone increased the percentage of CD34+CD38- cells, representing a primitive stem cell population (PSCP). RIGO alone, and RIGO/AZA SC, impacts different progenitors. Moreover, we found a remarkable reduction in colony forming unit number in response to RIGO (83%) and RIGO/AZA (90%).

Conclusions: Altogether results indicate RIGO appears to promote maintenance of a PSCP, while the RIGO/AZA SC appears to push the cells toward a cycling stage with increased expression of genes associated with OXPHOS. In comparison, when treated with RIGO, cells remain in a less differentiated stage. Studies are underway to determine the linkage of these pathways with hematopoiesis and the immune landscape.
STUDY OF ANGIOGENESIS GENE EXPRESSION AND BIOMARKERS IN MYELODYSPLASTIC SYNDROME AND CYTOPENIAS

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Background And Aims: The microenvironment of bone marrow can influence disease profile in Myelodysplastic Syndrome (MDS). Aim: to evaluate the expression of the genes and cytokines in patients with MDS, Idiopathic Cytopenia of Undetermined Significance (ICUS), Clonal Cytopenia of Undetermined Significance (CCUS) and Cytopenia Non-Neoplastic Cause (CNN).

Methods: Analysis were performed by RT PCR for gene expression and enzimaimmuno assay for bone marrow plasma biomarkers

Results: 72 patients was enrolled: MDS (n = 34), ICUS / CCUS (n = 31) and CNN (n = 7) HIF1-α gene was more expressed in CNN group (p = 0.019). IFNᵧ levels were also higher in CNN group (p = 0.017). Patients with fibrosis had higher levels of TNFα (p = 0.031) and IL-6 (p = 0.001). In MDS group, patients with advanced disease presented higher concentrations of TNFα (p = 0.045). IFNᵧ was higher in the initial stages (p = 0.003). There was a positive correlation between VEGFA and HIF1α gene (R = 0.834;p<0.001); HIF1α and TP53 gene (R = 0.595;p<0.001); VEGFA and TP53 gene (R = 0.551;p<0.001); HIF1α gene and IFN cytokine (R = 0.628;p<0.001); HIF1α gene and VEGFA cytokine (R = 0.502;p<0.001) for all groups. In MDS group the correlation was between VEGFA and HIF1α genes (R = 0.685;p<0.001)

Conclusions: Patients from CNN presented higher gene expression and cytokines levels. The reduced levels of IFNᵧ in the most advanced stage of SMD (RAEB-1 and RAEB-2) may suggest a deficient or insufficient production of functionally competent NK cells, which contributes to the progression of the disease through immune escape.
2-HG ENANTIOMERS IN MDS CLINICAL COURSE AND PROGNOSIS

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1National and Kapodistrian University of Athens, Dept. Of Pathophysiology, Athens, Greece, 2National Hellenic Research Foundation, Institute Of Chemical Biology, Athens, Greece, 3National and Kapodistrian University of Athens, 2nd Dept. Of Internal Medicine, Athens, Greece, 4Universitat Pompeu Fabra, Research Programme On Biomedical Informatics (grib), Hospital Del Mar Medical Research Institute (imim), Department Of Experimental And Health Sciences, Barcelona, Spain

Background And Aims: An untargeted metabolomic analysis performed on the maturing myeloid lineage from MDS patient, unraveled two distinct metabolic groups depending on bone marrow (BM) blast percentage. Apart from their overall differences the two metabolotypes featured pronounced discrepancies in redox potential and mitochondrial (dys)function while sharing increased 2-Hydroxyglutarate (2-HG) levels. Given the distinct origins and properties of the 2-HG enantiomers herein, we aim to shed light on the link of D-2-HG and/or L-2-HG levels with MDS metabolotypes and gain clinical insights.

Methods: Isolated bone marrow differentiating myeloid lineage (>95% purity, <1% blast contamination) from MDS patients with <5% (No.11) and >5% (No.4) intra-BM blasts and aged matched controls (No.7) were subjected to untargeted mass spectrometry-based metabolomics analysis. D-2-HG and L-2-HG levels were thereafter differentially determined.

Results: Both groups shared increased 2-HG levels. Metabolomic analysis of all samples featured a strong link between 2-HG levels, both enantiomers, and mitochondrial Complex IV abnormal function. D-2-HG has a known Complex IV inhibitory action while L-2-HG is supposed to act as a source of cellular reducing equivalents. Accordingly, the <5% intra BM blast group showed dominant mitochondrial dysfunction compatible with the D-2-HG inhibitory action on complex IV while the >5% group presented improved redox and respiratory potential despite the phenomenal electrontransport chain blockage both
compatible to a primarily L-2-HG driven profile.

**Conclusions:** Our findings underscore an etiologic relationship between the 2-HG enantiomer, mitochondrial (dys)function and redox state while justifying further research on this oncometabolite as a prognostic MDS biomarker.
MECHANISMS OF RESISTANCE TO THE HYPOMETHYLATING AGENT AZACITIDINE IN MYELODYSPLASTIC SYNDROMES: FOCUS ON METHYLATION

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Background And Aims: Hypomethylating agents (HMA) are standard of care for Myelodysplastic syndromes (MDS) but, still only 50% of patients have a clinical response and all of them will lose response. In this scenario, we aimed to investigate how and why some patients are primary resistant to azacitidine (AZA) therapy while others initially respond and then relapse.

Methods: We evaluated 22 cases of high-risk MDS, treated with AZA (75 mg/m²/d for 7 days every 28 days). Bone marrow aspirates were collected before and after treatment with the drug. For some cases, samples at baseline, at remission and at relapse were available. DNA methylation in CD34+ cells were investigated by ERRBS.

Results: Complete methylation analysis is available for three sets of paired samples. 25,538 (Baseline vs Post treatment), 4,010 (Post treatment vs relapse), 127 (Baseline vs relapse) differentially methylated regions (DMRs) were identified. The majority of DMRs localize in intergenic and intronic regions. After treatment with AZA, global genome DNA methylation decreased due to the widespread hypomethylating effect of the drug, while at relapse methylation increased in specific genomic regions. Some DMRs gained methylation at relapse and were different from the baseline ones indicating a reprogramming of methylation in CD34+ cells. Gene Ontology annotation of DMR-related genes reveals an enrichment in biological process related to neutrophil and granulocyte pathways.

Conclusions: This finding is important because for the first time a methylation analysis has identified in relapsed cases that loss of response to AZA could be caused by emerging reprogrammed clones.
RELATION BETWEEN IMMUNE CELL POPULATIONS AND MALIGNANT CLONE MUTATIONAL STATUS IN MYELODYSPLASTIC SYNDROMES

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¹Vall d’Hebron hospital/ University autonoma of Barcelona (UAB), Hematology, Barcelona, Spain, ²Experimental Hematology Unit, Vall d’Hebron Institute of Oncology (VHIO), Hematology, Barcelona, Spain, ³Vall d’Hebron hospital, Hematology, Barcelona, Spain, ⁴IBSAL university hospital, Laboratory, Salamanca, Spain, ⁵Centro de Investigación Médica Aplicada (CIMA), Navarra university-IDISNA., Oncology-hematology, Pamplona, Spain, ⁶Oncology Data Science (ODysSey) Group, Vall d’Hebron Institute of Oncology (VHIO), Data Science, Barcelona, Spain

Background And Aims: The role of immune dysregulations and somatic gene mutations are known prognostics factors in myelodysplastic syndromes (MDS). However, the importance of the interaction between them in the disease course is not entirely understood. This study aimed to characterize how the microenvironment regulates the malignant clone advantage.

Methods: We prospectively studied 40 MDS patients, 12 idiopathic cytopenia of unknown significance (ICUS), and 4 healthy donors (HD). We evaluated in bone marrow, by flow cytometry the following populations: natural killers (NK) (CD3CD56+CD16+/CD56+CD16−/CD56−CD16+); myeloid-derived suppressor cells (MDSC), granulocytic (Gr-MDSC) (CD11b+CD33+HLA-DR−CD15+CD14−) and monocytic (Mo-MDSC) (CD11b+CD33+HLA-DR+CD15+CD14+). T cells subpopulations were studied in peripheral blood (CD3/CD4/CD8/CCR7/CD45RA/CD27/CD27/CD57/CXCR3/CCR6). Molecular analysis included 40 genes associated with myeloid malignancies by NGS using the Oncomine Myeloid Research Assay (ThermoFisher Scientific).

Results: Table 1 shows patients characteristics. We observed a wide distribution of the different immune cells among patients (figure 1). In comparison with ICUS and HD, we found an increase of TCD4+PD1+ in ICUS vs MDS (p=0.022) and a decrease of effector TCD8+ (p= 0.02); and TNK (p=0.044) in MDS vs ICUS. Also, we detected a trend to decrease in CD56dimCD16+ (p=0.104) and an increased in MDSC, especially Mo-MDSC (p=0.103), in MDS (figure 2). Finally, not significant immune population changes were observed between different mutational
### Table 1 - Characteristics of MDS patients

<table>
<thead>
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<th>MDS patients</th>
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<tbody>
<tr>
<td>Number</td>
<td>40</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>81 (71.5-85)</td>
</tr>
<tr>
<td>Sex</td>
<td>21 (52.5%) M - 19 (47.5%) F</td>
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<th>Classification of WHO 2017</th>
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<tr>
<td>MDS-SLD</td>
<td>0</td>
<td>(0%)</td>
</tr>
<tr>
<td>MDS-MLD</td>
<td>27</td>
<td>(67.5%)</td>
</tr>
<tr>
<td>MDS EB-1</td>
<td>7</td>
<td>(17.5%)</td>
</tr>
<tr>
<td>MDS EB-2</td>
<td>6</td>
<td>(15%)</td>
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<th>IPSS-R group</th>
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<tr>
<td>Very low</td>
<td>5</td>
<td>(12.5%)</td>
</tr>
<tr>
<td>Low</td>
<td>17</td>
<td>(42.5%)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>11</td>
<td>(27.5%)</td>
</tr>
<tr>
<td>High</td>
<td>0</td>
<td>(0%)</td>
</tr>
<tr>
<td>Very high</td>
<td>7</td>
<td>(17.5%)</td>
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<table>
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<tr>
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<tr>
<td>Normal</td>
<td>24</td>
<td>(60%)</td>
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<tr>
<td>Abnormal</td>
<td>16</td>
<td>(40%)</td>
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<th>Cytogenetic risk</th>
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<tr>
<td>Very Good</td>
<td>4</td>
<td>(10%)</td>
</tr>
<tr>
<td>Good</td>
<td>23</td>
<td>(57.5%)</td>
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<tr>
<td>Intermediate</td>
<td>7</td>
<td>(17.5%)</td>
</tr>
<tr>
<td>Poor</td>
<td>0</td>
<td>(0%)</td>
</tr>
<tr>
<td>Very poor</td>
<td>5</td>
<td>(12.5%)</td>
</tr>
</tbody>
</table>

MDS-SLD myelodysplastic syndrome with single-lineage dysplasia, MDS-MLD MDS with multilineage dysplasia, MDS EB-1, -2 myelodysplastic syndrome with excess blasts-1, -2. IPSS-R revised international prognostic scoring system.
Figure 1. Immune populations
95% CI for the Mean

Percentage (%)

Group
HD
ICUS
MDS

T CD3
CD3+CD4+
CD3+CD8+
Tregs
Th1
Th2
Th17
Th1-17
PD1
TNK
CD16+ CD56dim
Conclusions: Immune populations show a very heterogenic pattern among MDS patients, with a trend towards decreasing cytotoxic profile. No relationship was found with the mutational status. Further results to increase the sample are ongoing.
PHENOTYPE AND GENOTYPE OF NATURAL KILLERS IN MYELODISPLASITIC SYNDROME


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Background And Aims: Impaired cytotoxicity and a decrease in mature natural killers (NK) in myelodysplastic syndrome (MDS) have been described. Moreover, KIR haplotype A have been associated with disease progression. The aim of our study is to phenotypically analyze NK cells receptors and ligands and to evaluate the correlation with KIR haplotype.

Methods: We prospective studied bone marrow samples from 33 MDS patients, 12 idiopathic cytopenia of unknown significance (ICUS) and 4 healthy donors (HD). We define NK cells as CD3-CD56+CD16+; activating receptors (NKp46, NKp30, NKG2C, NKG2D, NKp44, DNAM) and inhibitory receptors (TIGIT, NKG2A, Irp60, PD1) and their ligands (HLA-ABC, MICA-B, CD155, PD-L1). KIR haplotype was analyzed by NGS.

Results: Table 1 shows patients characteristics. Compared to controls, MDS showed a decrease in CD56dimCD16+. Immunophenotypic characteristics showed a significant decrease in NKG2C (p=0.039) and KIR2DS4 (p=0.036) in MDS. No significant differences were found in inhibitory receptors. Ligands studies in MDS cells showed no significant loss of HLA, CD155 nor PD-L1 expression, but a significant loss in MIC-A/B expression was found in MDS vs. controls (p=0.034). Finally, no immunological profile differences in NK receptors between the different haplotypes, was found. After a median follow up of 9.74 months (IQ: 1.75-19), 3 (10%) patients presented disease progression and 5 (15%) died. Progression free survival between haplotypes, showed no differences (figure
## Table 1 - Characteristics of MDS patients

<table>
<thead>
<tr>
<th>MDS patients</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>73.3 (70.5-85)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>196 (58.9%) M - 142 (41.2%) F</td>
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<table>
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<tr>
<th>WHO 2017 MDS classification</th>
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<tbody>
<tr>
<td>MDS-SLD</td>
<td>0</td>
<td>(%)</td>
</tr>
<tr>
<td>MDS-MILD</td>
<td>22</td>
<td>(66.67%)</td>
</tr>
<tr>
<td>MDS EB-1</td>
<td>6</td>
<td>(18.78%)</td>
</tr>
<tr>
<td>MDS EB-2</td>
<td>5</td>
<td>(15.15%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IPSS-R group</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Very low</td>
<td>3</td>
<td>(9.09%)</td>
</tr>
<tr>
<td>Low</td>
<td>15</td>
<td>(45.45%)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>9</td>
<td>(27.27%)</td>
</tr>
<tr>
<td>High</td>
<td>0</td>
<td>(0%)</td>
</tr>
<tr>
<td>Very high</td>
<td>6</td>
<td>(18.68%)</td>
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<table>
<thead>
<tr>
<th>Karyotype</th>
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<tbody>
<tr>
<td>Normal</td>
<td>20</td>
<td>(60.61%)</td>
</tr>
<tr>
<td>Abnormal</td>
<td>13</td>
<td>(39.39%)</td>
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<table>
<thead>
<tr>
<th>Cytogenetic risk</th>
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<tbody>
<tr>
<td>Very Good</td>
<td>4</td>
<td>(12.12%)</td>
</tr>
<tr>
<td>Good</td>
<td>20</td>
<td>(60.81%)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>5</td>
<td>(15.15%)</td>
</tr>
<tr>
<td>Poor</td>
<td>0</td>
<td>(0%)</td>
</tr>
<tr>
<td>Very poor</td>
<td>4</td>
<td>(12.12%)</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>KIR haplotype</th>
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<tbody>
<tr>
<td>Haplotype A</td>
<td>10</td>
<td>(30.3%)</td>
</tr>
<tr>
<td>Haplotype B</td>
<td>23</td>
<td>(69.7%)</td>
</tr>
</tbody>
</table>

Conclusions: A decrease in NK cells activating receptors was observed in MDS, regardless of KIR haplotype. Additionally, we observed a loss in MICA/B expression in MDS.
HIGH-DIMENSIONAL PROFILING OF T AND NK CELL FREQUENCIES AND UNSUPERVISED CLUSTERING ANALYSES TO IDENTIFY IMMUNE (DYS)FUNCTIONAL PHENOTYPES IN MDS PATIENTS

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Background And Aims: For MDS patients, especially those characterized as higher risk, allogeneic stem cell transplantation is the only curative treatment, which can be attributed to powerful donor-derived anti-tumor immune responses. However, patients are often ineligible due to high age and frailty. This emphasizes the need for adjuvant (immuno)therapies to prevent disease progression and improve overall survival. Insights into the level of immune (dys)function in MDS will provide directions to boost tumor-reactive T and NK cells.

Methods: Here, high-dimensional flow cytometry and unsupervised clustering analyses (i.e. FlowSOM) were used to examine the bone marrow of 10 healthy donors and 30 MDS patients in-depth, with a specific focus on T and NK cells.

Results: Our results corroborate with literature, displaying higher levels of regulatory T cells in higher risk MDS, and lower abundance of naïve-like CD8⁺ T cells in all MDS groups. Moreover, within the CD8⁺ T cells, an increase in subpopulations reflecting terminal differentiation (CD45RA⁺CCR7⁻KLRG1⁺), exhaustion (PD-1⁺,TIGIT⁺) and senescence (CD57⁺) was observed. Furthermore, we found an enrichment of CD4⁺CD39⁺ clusters that co-expressed multiple co-inhibitory markers. CD39 is of specific interest, as it converts ATP to the immunomodulatory adenosine and has been linked to tumor-specificity. Similarly, NK cell subsets displaying activating receptors (i.e. NKp30⁺, DNAM⁺, NKG2A⁺) were decreased in higher risk MDS. Interestingly, T cells from MDS patients displayed poly-functionality, increased degranulation potential and increased IL-2, IFNy and TNFα production, independent of memory phenotype.

Conclusions: By integrating phenotypic profiles with functional data we acquire insight into the level and complexity of T and NK cell (dys)function in MDS.
E-POSTER VIEWING

T-CELL RECEPTOR SEQUENCING AND T-CELL MONOCOLONALITY: A NOVEL BIOMARKER OF RESPONSE TO IVIG IN MDS PATIENTS WITH AUTOIMMUNE CYTOPENIAS

Icahn School of Medicine at Mount Sinai, Hematology And Medical Oncology, New York, United States of America

Background And Aims: We’ve reported clinical responses in MDS patients with autoimmune cytopenias and T-cell clonality treated with intravenous immunoglobulin (IVIG). T-cell clonality was previously assessed via gel-based testing of TCR beta (TRB) and/or gamma (TRG) gene rearrangements. NGS of TRG and TRB (TCR-Seq) offers superior resolution in identifying T-cell clones.

Methods: We processed the peripheral blood of 9 patients (out of 27 previously treated with IVIG +/- steroids) who had serial samples available. Clinical responses were per MDS IWG 2006 criteria. TCR-Seq was performed using the Lymphotrack® TRG and TRB Assays on Illumina® MiSeq platform (Invivoscribe, Inc.). T-cell clonality was interpreted based on percent total merged clonal reads and polyclonal background.

Results: By IPSS criteria, six patients were intermediate-1 and one was low risk. All patients showed T-cell clonality by gel PCR; 100% were TRB+ and 7 were TRG+. Of four patients who were non-monoclonal by TCR-Seq, only one had an erythroid response. Hemolytic responses were mostly minimal as well. Median duration of treatment was 1.5 months. Of five patients who had a monoclonal TRB and/or TRG population by TCR-Seq, 80% had hematologic improvement with more significant hemolytic responses. Median duration of treatment was 45 months. Three of four patients had reductions in their clonal burden during treatment.

Conclusions: TCR-Seq is a superior assay to PCR-based techniques for T-cell clonality, can track clonal burden over time, and in a small subset of patients, those who displayed T-cell monoclonality had improved responses. It may therefore prove to be a useful biomarker of response to IVIG.
ABERRANT SMAD2/3 SIGNALING IN MDS BONE MARROW STROMA CONTRIBUTES TO ALTERED ECM PROTEIN DEPOSITION AND CAN BE RESCUED BY LUSPATERCEPT

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Background And Aims: The involvement of the bone marrow microenvironment (BMME) into myelodysplastic syndrome (MDS) progression and therapeutic response is indisputable. It provides an efficient milieu for hematopoiesis and may be targeted by clinically available drugs such as luspatercept, a TGFβ superfamily ligand trap. Although the cellular BMME components have been increasingly investigated, the extracellular matrix (ECM) is poorly studied so far and was therefore investigated here.

Methods: We compared structural and functional characteristics of ECM from mesenchymal stromal cells (MSCs) of healthy donors (n=5) and serial samples (pre- and post-treatment with luspatercept) of age-matched low-risk MDS patients carrying SF3B1 mutation and ring sideroblasts (n=6).

Results: Scanning electron and atomic force microscopy displayed a more compact and significantly thicker but softer ultrastructure of MDS ECM than those from healthy MSCs. This was accompanied by significantly higher levels of sulfated GAGs in MDS ECM as measured by Blyscan assay (4.6µg vs. 1.1µg/cm²). Moreover, immunofluorescence staining and Western blot demonstrated increased appearance of collagen I/IV. Interestingly, inhibition of Smad2/3 signaling by luspatercept modulated the ECM characteristics towards less compact structure and lower collagen levels. This led to advanced hematopoietic support with higher expansion rates of CD34+ cells and increased clonogenic potential. Moreover, HSPCs cultured on matrices from luspatercept-treated MDS MSCs displayed a more polarized cytoskeleton and accumulation of integrin avb3 suggesting an improved interaction with the ECM as this is the case for healthy MSC-derived ECM.

Conclusions: Modification of endogenously altered MDS ECM by luspatercept provides an additional mode of action of this agent in LR-MDS treatment.
P35 / #190

Topic: AS04-MDS Biology and Pathogenesis / AS04i-Microenvironment and stem cell niche

E-POSTER VIEWING

PATIENTS DIAGNOSED WITH MYELODYSPLASTIC SYNDROMES HAVE LOWER BONE MASS

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Background And Aims: Murine models of Myelodysplastic syndromes (MDS) exhibit lower bone mass (BMass), and several reports suggest increased incidence of osteoporosis and fractures in MDS patients. Whether MDS is associated with lower BMass is unknown. Aims: 1) to develop a simple method to estimate trabecular BMass from bone marrow biopsies (BMB). 2) to compare the trabecular BMass of MDS patients at diagnosis and non-MDS controls.

Methods: Patients. BMB of Men ≥65 years with "lower-risk" (LR) MDS diagnosed at Tel Aviv Sourasky Medical Center, between 2011 and 2019 and age-matched controls undergoing BMB for unexplained anemia. BM slides (H&E stain) were digitally scanned. The total relevant area (TRA, figure 1A) was identified, and bone trabeculae were manually contoured (Adobe Photoshop). Bone area (BA, figure 1B) was the sum of all contoured pixels of bone. BMass = BA/TRA(%) .

Figure 1: (A) TRA (B) BA

Results: There were 43 MDS and 36 control patients. Mean ages (80 vs 78 years, p=0.07) and comorbidities in both groups were similar; MDS patients had lower blood counts. BMass of MDS and controls were 11.6% [95%CI 9.9-13.3] vs 18.3% [16.6-20.3], respectively (p<0.0001), representing a 37% relative reduction in BMass for MDS patients.
Conclusions: 1) We developed a simple technique to estimate trabecular BMass in MDS (and other) patients, based on available BM sections. 2) Trabecular BMass of LR-MDS patients is compromised already at diagnosis. Future studies will evaluate the clinical significance of this finding in MDS.
MESENCHYMAL STROMAL CELLS MAY ENHANCE RESIDUAL HEALTHY HAEMATOPOIESIS IN LOW-RISK MDS PATIENTS

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1Hospital Universitario de Salamanca - IBSAL, Hematology, Salamanca, Spain, 2Cancer Research Center of Salamanca, Hematology, Salamanca, Spain, 3Instituto Maimonides de Investigación Biomédica de Córdoba, Biología Celular En Hematología, Córdoba, Spain, 4Centro de Investigación Médica Aplicada (CIMA), Navarra university-IDISNA., Oncology-hematology, Pamplona, Spain, 5Centro de Investigación Médica Aplicada (CIMA), Navarra university-IDISNA., Hematology, Pamplona, Spain, 6Vall d’Hebron hospital/ University autonoma of Barcelona (UAB), Hematology, Barcelona, Spain, 7Experimental Hematology Unit, Vall d’Hebron Institute of Oncology (VHIO), Hematology, Barcelona, Spain

**Background And Aims:** Mesenchymal stromal cells (MSC) in MDS patients are characterised by poor hematopoietic support capacity. Considering its involvement in the pathophysiology of MDS, we aimed to study whether healthy MSC might have therapeutic potential in the positive regulation of residual healthy hematopoiesis in low-risk MDS patients.

**Methods:** Bone marrow samples from low-risk MDS patients (n=21) [WHO 2017: MDS-MLD category (IPSS-R: very low to intermediate)], newly diagnosed, as well as from healthy donors (HD) (n=13), were analysed. Mononuclear cells (MNC) and MSC were isolated and expanded according to standard methodology. A short-term transwell co-culture model was established (Fig. 1). After 48h, clonogenic assays were performed on methylcellulose medium with EPO from MNC of each group. After 14 days, CFU-erythroid (CFU-E) and CFU-granulomonocytic (CFU-GM) were counted (CFU/5000 MNC seeded).

![Image of the short-term transwell co-culture model](image)

*Figure 1. Short-term transwell co-culture model (48h; MNC:MSC ratio of 3:1), with different cell combinations.*

MDS-MNC: mononuclear cells from MDS patients, MDS-MSC: mesenchymal cells from MDS patients, HD-MNC: mononuclear cells from healthy donors, HD-MSC: mesenchymal cells from healthy donors, MNC Mix: combination of MDS-MNC and HD-MNC, Control MDS-MNC and HD-MNC: MNC in basal state, not co-cultured with MSC.
Results: Basal MDS-MNC showed a deficient formation of CFU-E and CFU-GM compared to HD-MNC. Ratio between CFU formed by MDS-MNC co-cultured with MSC (MDS or HD) versus basal MDS-MNC (groups 1.1 and 2.1), showed that the addition of HD-MSC trends to increase CFU-E formation (Fig. 2-A) and significantly enhances CFU-GM (Fig. 2-B). Simulating the disease condition (groups 3 and 4) with mixed MDS and HD MNC (dysplastic and residual healthy hematopoiesis), demonstrated a significant recovery of hematopoietic capacity with the addition of MSC, greater if HD-MSC (Fig. 2, A and B).

Conclusions: Our results suggest that HD-MSC enhances MNC clonogenic capacity, especially when co-cultured with mixed MDS and HD MNC, and could be a potential therapy to rescue residual healthy hematopoiesis in LR-MDS patients. Funding: PI17/01741, CM17/00171
A NOVEL SCORING SYSTEM INTEGRATING MOLECULAR ABNORMALITIES WITH IPSS-R CAN IMPROVE THE RISK STRATIFICATION IN PATIENTS WITH MDS

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Zhongda Hospital, School of Medicine, Southeast University, Institute of Hematology Southeast University, Department Of Hematology, Nanjing, China

Background And Aims: We aimed to establish a novel risk stratification for MDS based on the revised international prognostic scoring system (IPSS-R) with gene mutations that can be easily applied in the real world.

Methods: The training cohort of 63 MDS patients was conducted at Zhongda Hospital of Southeast University from January 2013 to April 2020. The validation cohort of 141 MDS patients was obtained from GSE129828. The mutation scoring system was based on the number of mutations and a unique favorable prognostic factor SF3B1 mutation.

Results: A novel risk scoring system named “MIPSS-R” was developed based on the results derived from multivariate analysis which assigned points to the IPSS-R and the mutation scores according to their relative statistical weight. Based on the quintile of MIPSS-R scores, patients were divided into five risk levels. The Kaplan-Meier curves showed the superiority of MIPSS-R in separating patients from different groups, comparing with IPSS-R both in the training cohort and validation cohort. The area under the ROC of MIPSS-R was 0.79 in the training cohort and 0.62 in the validation cohort. The retrospective analysis of our house patients showed that the risk levels of 57.41% of patients would adjust according to MIPSS-R. After changing risk levels, 38.71% of patients would be benefitted from treatment strategies that MIPSS-R recommends.
Conclusions: A mutation scoring system was conducted based on the number of mutations and a unique favorable prognostic factor. MIPSS-R was developed by integrating IPSS-R and the mutation scores, which is more effective on prognosis and treatment guidance.
NEW SCORE PROGNOSIS BASED ON SOMATIC MUTATIONS BY NGS AND THE NUMBER OF BLASTS FOR PREDICTING OVERALL SURVIVAL IN PATIENTS WITH MYELODYSPLASTIC SYNDROME.

M. Poza¹, A. Ruiz-Medina², J. Martinez-Lopez¹,³,⁴ I. Rapado¹,⁵, M.T. Cedena¹,⁵,⁶, R. Ayala¹,³,⁵
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Background And Aims: Myelodysplastic syndrome (MDS) prognostic classification is mainly based on cytogenetics, but in recent years somatic mutations have also become important. This study aims to create a new score based on somatic mutations detected by next generation sequencing (NGS) techniques.

Methods: This is an observational study of 147 patients with MDS diagnosed at Hospital 12 de Octubre. Molecular studies by NGS were performed with Ion Torrent Proton® platform, and 56 genes related to myeloid pathology were examined. Impact of somatic mutations on overall survival (OS) was investigated by Cox regression in univariate and multivariate analysis. Finally, we developed a prognostic index considering the main molecular alterations and their prognostic value.

Results: 133 of the 147 patients (90.5%) harbored at least one mutation, with a median of 2 (0–7). The most frequently mutated genes were TET2, SF3B1, DNMT3A, ASXL1 and RUNX1.
Mutations in seven genes predicted lower OS, and only SF3B1 mutations were associated with better outcomes. A three-level risk index was developed based on the prognostic impact provided by each mutation and the percentage of blasts in the bone marrow. Mutations in ASXL1 and ETV6 were included due to their prognostic value demonstrated in previous publications and in which statistical significance was not obtained, probably because of the lower frequency. This classification was also applicable in patients who only received supportive treatment and in those with low or intermediate risk cytogenetics.
Conclusions: This score could be complementary to the commonly used indexes, although validation of the results in large external cohorts is necessary.
ADDITIONAL ADVERSE PROGNOSTIC IMPACT OF GENETIC MUTATIONS IN THE HIGH-RISK GROUP PATIENTS WITH CMML

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Hospital Universitario 12 Octubre, Hematology, Madrid, Spain

Background And Aims: Molecular markers seem influence the pathogenesis and progression of chronic myelomonocytic leukemia (CMML). Our aim is to find out whether molecular markers add prognostic value to the usual prognostic score.

Methods: We analysed the mutational profile of 23 CMML patients, by next generation sequencing (NGS). Additionally, CMML-prognostic scoring (CPSS), and outcome were considered. The statistical analyses were conducted with SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). Cox proportional hazard models and Kaplan-Meier curves were performed.

Results: Twenty-three CMML patients were analysed (median age: 75). Mutations were found in all patients, with a median of 2 (1-4). The most frequent were in: TET2(70%), KRAS(13%), EZH2(13%), CBL(13%), ASXL1(9%), NRAS(9%), RUNX1(9%). According to the CPSS prognostic score, differences in survival were found between the low-intermediate1 risk group (median: 48 months) and the intermediate2-high risk group (median: 10 months); (p=0.011) [Figure 1].
Therefore, we considered CPSS and molecular risk (defining high molecular risk as the presence of at least one mutation in ASXL1, RUNX1, NRAS, or KRAS), and established three risk categories: low risk (CPSS low-intermediate1 AND low molecular risk), intermediate risk (CPSS intermediate2-high AND low molecular risk) and high risk (high molecular risk). The prognosis is particularly adverse in the high-risk
group, with a poor survival (median: 9 months); ($p=0.044$) [Figure 2].

Conclusions: In the intermediate-2 and high-risk group of CPSS, those patients with mutations in high-risk genes (ASXL1, RUNX1, NRAS or KRAS) have a significant adverse prognosis of less than 1 year.
BEST BETTER THERAPEUTIC APPROACH AFTER COMPREHENSIVE GERIATRIC ASSESSMENT IN OLDER PATIENTS WITH MYELODYSPLASTIC SYNDROME.

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Background And Aims: Most patients with myelodysplastic syndromes (MDS) are older and physicians are often uncertain about how to identify pts who may benefit from specific treatment strategies. Comprehensive geriatric assessment (CGA) is considered the gold standard tool to classify pts according to frailty profile. Multidisciplinary approach including a geriatrician is essential. CGA may be helpful to personalize treatment plan and to detect conditions that can be reversible through geriatric interventions. We aim to evaluate the impact of CGA on therapeutic decisions in pts with MDS.

Methods: From January 2018 to March 2021, 52 pts with MDS were evaluated systematically with a CGA which include validated instruments to assess comorbidity, polypharmacy, functional status, geriatric syndromes, mood, cognition and social status. According to CGA pts were classified in 3 frailty groups: fit, medium fit and unfit.

Results: Median age was 77 years (range 67-90). 63% males. Diagnosis: 52% MDS, 21% CMML and 27% AML <30% blasts. Among MDS, R-IPSS categories: 4% very low, 21% low, 24% intermediate, 19% high and 17% very high risk. 44% of pts were transfusion dependent. Based on CGA, 51% were classified as fit, 36% medium fit and 11% unfit. Regarding CGA, 44% of pts need intervention: 16% physiotherapy, 24% psychological support, 32% nutritional, 4% polypharmacy, 18% social work and 4% palliative intervention. Patients therapeutic approach was: 16% Clinical Trial, 51% Azacitidine, 10% chemotherapy, 8% target therapy and 15% exclusive supportive care.

Conclusions: Incorporation of CGA within multidisciplinary approach brings the opportunity to better classify pts according to frailty profiles so as to guide interventions and treatment decisions.
PROGNOSTIC SIGNIFICANCE OF COMORBIDITIES AND FRAILTY IN RISK STRATIFICATION OF PATIENTS WITH MYELODYSPLASTIC SYNDROME

M. Efraim, I. Micheva
UMHAT"St. Marina", Hematology, Varna, Bulgaria

Background And Aims: Background: Myelodysplastic syndrome (MDS) is a heterogeneous group of clonal disorders resulting in bone marrow failure with dysplasia in one or more lineages, cytopenia and high risk of transformation into acute myeloid leukemia. Disease-related factors are included in the established prognostic scoring systems - International Prognostic Scoring System (IPSS), revised – IPSS (IPSS-R), WHO prognostic scoring system (WPSS). The addition of patient-related factors such as frailty and comorbidities can improve prognostication. Aim: To assess and incorporate frailty (Clinical frailty scale (CFS)) and comorbidities (Charlson comorbidity index (CCI), Hematopoietic stem cell-comorbidity index (HCT-CI), MDS-comorbidity index (MDS-CI), Adult comorbidity evaluation-27 (ACE-27)) within the IPSS, IPSS-R and WPSS for risk stratification and survival.

Methods: Materials and methods: We applied CFS, CCI, HCT-CI, MDS-CI and ACE-27 in 219 patients with MDS, diagnosed and treated in the hematology clinic of UMHAT "St. Marina" Varna, Bulgaria between May 2010 and May 2020. IPSS, IPSS-R and WPSS were used for prognostic stratification. Statistical analysis was performed using SPSSv.20.

Results: Results: The mean age of patients was 70.7±10.2 years. The mean survival was 18.4±21.9 months. 56.2% of patients had comorbid conditions. Patients with comorbidities ≥1 and CFS>3 had significantly poorer prognosis and worse outcome (p<0.001). We found significant difference in mean survival according to the comorbidity indexes and CFS independently of IPSS, IPSS-R and WPSS (p<0.001).

Conclusions: CFS and comorbidities provide prognostic value to the established prognostic systems. They are significant and independent determinants for survival. Incorporation of comorbidity index into existing prognostic scoring systems may improve prognostication in MDS.
LYMPHOID AGGREGATES IN MDS BONE MARROW BIOPSIES – A PROGNOSTIC MARKER?

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Background And Aims: Clusters of lymphoid aggregates (LA) are occasionally observed in bone marrow biopsies (BMB) of patients without lymphoproliferative disorders, particularly with myelodysplastic syndromes (MDS). Our aim was to evaluate their incidence and their association with MDS prognosis.

Methods: We retrospectively compared baseline BMB reports of MDS patients (Tel Aviv Sourasky Medical Center) between 2011-2018, to controls (2017-2018) with reported normal BMB (same pathologist). We then examined the charts of MDS patients with (LA+) and without LA (LA-).

Results: In total, 178 patients (MDS=140; controls=38) were included. MDS patients were older (74.1 vs 69.2 years, p=0.005); gender distribution was similar. Of the MDS group, 34 patients (24.3%) had LA+, 106 did not. In the control group, only 5 patients (13.2%) had LA. CD20 and CD3 staining, where performed, demonstrated the polyclonality of LA. MDS LA+ (vs LA-) patients were younger, with trends of poor parameters: lower Hb, WBC, and platelets, higher LDH, BM cellularity, blast% and IPPS/IPSS-R prognostic scores (P>0.05 all). The incidence of cardiovascular disease and cancer was similar but diabetes was common in MDS LA+ (38.2% vs 19.0%, p=0.022). At 1 year, 24/34 (70.6%) MDS LA+ and 88/106 (83.0%) MDS LA- patients were alive (p=0.098). At 2 years, 23/34 (67%) and 73/106 (68.9%, p=0.692), respectively.

Conclusions: Such preliminary data suggest with caution that LA might indicate poor prognosis in MDS. This may reflect involvement of the immune system in MDS pathogenesis. Future studies will examine larger groups, to clarify the incidence, significance and the biology of these findings.
HIGH-RISK IMMUNOPHENOTYPIC MARKERS IN PATIENTS WITH MYELODYSPLASTIC SYNDROME

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Background And Aims: Immunophenotypic examination of bone marrow cells is relevant for the diagnosis of MDS and the subsequent assessment of the result of the therapy. The aim of the study was to identify unfavorable prognostic immunophenotypic markers of blast cells in patients with high-risk myelodysplastic syndrome.

Methods: The prospective cohort study included patients with newly diagnosed MDS between January 2017 and April 2021. The patients underwent primary diagnostic MDS complex (including immunophenotyping of bone marrow aspirate). To explore immunophenotypic markers we use three state “illness-death” model. We consider the following states: diagnosis, transformation into leukemia; death.

Results: We identified high-risk immunophenotypic markers for transition “diagnosis-death”: CD38 <50% HR 3.7 (1.2-11.5 CI; p = 0.022), CD13> 50% HR 8.7 (1.1-67.8 CI; p = 0.04); for transition “diagnosis-transformation”: CD71 ≥65% HR 4.1 (1.3-12.4 CI; p = 0.013); CD13> 75% HR 2.8 (1.1-7.1 CI; p = 0.034); for transition “transformation-death”: CD25 <5% HR 6.3 (1.4-28 / 4 CI; p = 0.017), CD 33 <50% HR 6.6 (1.3-34.7 CI; p = 0.026).

Conclusions: The selection of groups of patients with identified immunophenotypic markers of a high risk of transformation into acute leukemia and a high risk of death raises the question of clinicians developing a new approach to the treatment of this group of patients.
Topic: AS06- Prognosis / AS06a- Prognostic factors of outcome and risk assessment

E-POSTER VIEWING

PROGNOSTIC SIGNIFICANCE OF HISTOLOGICAL BLAST COUNT IN MYELODYSPLASTIC SYNDROMES

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Background And Aims: Cytological assessment of the blast cell count is a gold standard in the diagnostics of MDS. However, aspirates are not always available. We aimed to evaluate the role of histological examination in the MDS risk prediction.

Methods: We retrospectively analyzed 383 patients (median age 65 years, m/f = 222/161), registered 2010-2020 in Dresden MDS Register. Data from 287 patients were sufficient for IPSS-(R) calculation. Blast count from histology was recorded as minimum, maximum and mean values. Based on the blast count from cytology and histology, we calculated IPSS-(R), IPSS-(R)HistoMax and IPSS-(R)HistoMean and compared the prognostic value of survival endpoints using Akaike’s information criterion (AIC) of Cox regression models.

Results: Mean blast count from cytology /HistoMax/HistoMean in the IPSS risk groups was: LOW - 2.9/4.2/2.1%, INT-1 – 4.8/5.0/ 2.6%, INT-2 – 11.3 /5.9/3.1%, HIGH – 14.5 /10.1 /6.0%. IPSS-R: VERY LOW – 1.4/4.0/2.0%, LOW – 3.2/4.4/2.2%, INT - 6.1/4.7/2.4%, HIGH - 9.6/6.5/3.6%, VERY HIGH – 11.2 /8.1/4.7%. AIC for overall survival for IPSS-(R), IPSS-(R) HistoMax and IPSS-(R) HistoMean was equivalent to 808.7 (807.6), 815.6 (809.5) and 809.9 (807.6). AIC regarding transition to AML was 468.0 (473.7), 468.8 (479.8) and 483.2 (483.8).

Conclusions: The histological blast count was consistently lower than the cytological blast count in all but lower risk patients, so that stratification by histology may lead to the risk underestimation. In the absence of cytology, risk assessment for OS by IPSS-RHistoMean and for AML progress by IPSSHistoMax appears to be a comparable alternative to the classic IPSS-R.
DEVELOPMENT OF NOVEL NASCENT RNA/ACTIVE CHROMATIN-DRIVEN FLOW IMAGING TECHNOLOGIES AND BIOMARKERS FOR PREDICTION OF DRUG RESISTANCE AND OPTIMIZATION OF DRUG COMBINATIONS IN MDS AND LEUKEMIA

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Methods: 5-ethynyl uridine (EU) clicking chemistry flow cytometry (EC-FLOW) and confocal microscopy with photon counting (EC-microscopy).

Results: 1. Development of novel EC-FLOW and EC-microscopy technologies for measurement and visualization of nascent RNA synthesis dynamics in a drug- and lineage-specific manner. 2. In spite of having more active RNAPII and co-factors, drug-resistant leukemia cells have unexpectedly slower rates of nascent RNA synthesis/transcription than drug-sensitive leukemia cells (Figure 1).

3. Our novel technologies and the newly identified drug- and lineage-specific TAC-based biomarkers could efficiently differentiate between drug-sensitive and drug-resistant leukemia cells (Figure 1).
Conclusions: We have developed novel EC-FLOW/EC-microcopy technologies and TAC-based drug-selective biomarkers, which provide practical tools and novel biomarkers for predication of drug resistance and optimization of drug combinations in clinical settings. Acknowledgement: The authors gratefully acknowledge the funding support from the Taub Foundation, the Michael Reese Bench to Bedside Award, and the CTSA-ITA Core Subsidies (#UL1TR002389) to J.X.C.
E-POSTER VIEWING

MOVING CLOSER TOWARDS EXPLAINABLE AI APPLICATIONS: DEVELOPMENT OF A SEMANTIC AND CAUSAL MODEL FOR PERSONALIZED TREATMENT DECISION MAKING IN MYELODYSPLASTIC SYNDROMES

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Background And Aims: While artificial intelligence (AI) can be implemented to offer assistance to physicians during clinical reasoning and decision-making processes, those require formalization of domain-specific medical knowledge to deduce meaningful therapy suggestions.

Methods: To generate a holistic representation model of personalized treatment pathways in MDS, we compiled all required individual parameters through extensive analysis of clinical practice guidelines, clinical case documentation (>2000) and published evidence. We then semantically defined each individual parameter through mapping onto a corresponding SNOMED-CT or LOINC concept before its integration into a HL7 FHIR resource for interoperable storage. We subsequently modeled the interaction and dependency of individual parameters, which are essential for the personalized treatment decision process, into a causal graph-based network using the “Resource Description Framework”.

Results: Based on our analysis to gather all information involved in the individual treatment decision process for MDS patients, we were able to identify and semantically describe a total of 303 individual parameters. Furthermore, a total of 2121 individual causal links between the parameters emerged, reflecting the immense complexity of the overarching decision problem.

Conclusions: We developed a semantic and causal model of treatment decisions in MDS that provides us with an extensive formal representation of the actual knowledge-based process. This newly developed model will be used to integrate methods for automatic reasoning of the presented decision problems to computationally solve the multi-factorial classification tasks. We aim to provide valid assistance for the complex cognitive processes needed for the development of a next-generation stratification and prognosis system and an AI-assisted clinical decision-support platform for MDS patients.
HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CHILDREN AND ADOLESCENTS WITH GATA2-RELATED MYELODYSPLASTIC SYNDROME


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Background And Aims: GATA2 deficiency is a heterogeneous multi-system disorder characterized by high risk of developing myelodysplastic syndrome (MDS) and myeloid leukemia. The aim of the study was to characterize the HSCT outcome of pediatric patients with MDS and GATA2 germline mutations (GATA2mut) and compared it to MDS without known underlying predisposition (GATA2wt).

Methods: Patients reported to the registry of the European Working Group (EWOG) of MDS in childhood were analyzed.

Results: Sixty-five patients were diagnosed with RCC (36), MDS-EB (22), MDS-EBt (6) or MDR-AML (1) at a median age of 12.8 yrs. Karyotypes included monosomy 7 (44), der (1;7) (4), trisomy 8 (4), random...
aberration (1) or normal karyotype (12). Forty patients (71%) had additional non-hematological features of GATA2 deficiency. HSCT was performed from a matched sibling donor (17), unrelated donor (40) or mismatched family donor (8). Patients were prepared with busulfan-based (35), treosulfan-based (21), irradiation-based (5) or alternative conditioning regimens (4). The probability of overall survival and disease-free survival (DFS) was 75% and 70%, respectively. Non-relapse mortality and relapse equally contributed to treatment failure. There was no evidence of increased incidence of graft-versus-host-disease or excessive rates of infections or organ toxicities. Advanced disease and monosomy 7 (-7) were associated with worse outcome. Comparing outcome of GATA2mut with GATA2wt patients, there was no difference in DFS in patients with RCC and normal karyotype and patients with -7 across morphological subtypes.

**Conclusions:** We demonstrate that HSCT outcome is independent of GATA2 germline mutations in pediatric MDS suggesting the application of standard MDS algorithms and protocols.
PROLONGED SURVIVAL OBSERVED IN 133 MDS PATIENTS TREATED WITH ORAL DECITABINE/CEDAZURIDINE


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Background And Aims: DNMTi are active in MDS treatment, however chronic parenteral therapy constitutes a burden for these patients, often elderly with co-morbid conditions. Oral decitabine(35 mg)/cedazuridine(100mg) given Days 1-5 every 28 days produces equivalent pharmacokinetic exposure (AUV) to 20 mg/m² IV decitabine dosing (Garcia-Manero, ASH 2019).

Methods: This randomized, cross-over study enrolled MDS/CMML subjects appropriate to receive IV decitabine per the US label. Subjects either received IV decitabine or oral decitabine/cedazuridine, followed by the converse in C2, allowing intrapatient PK comparison. All subjects received oral decitabine/cedazuridine for subsequent cycles providing longer term safety and efficacy data.

Results: 133 patients (IPSS HR: 16%, Int-1: 48%, Int-2: 20%, LR:4%, CMML:12%) were enrolled (US and Canada). The median age was 71y; 65% Male; 41% RBC and 9% platelet transfusion dependent,
respectively. Subjects received a median of 9 cycles of treatment and 26% proceeded to HCT, typically after 4-6 cycles. The most common adverse events of thrombocytopenia, neutropenia, and anemia were consistent with expected AEs with parenteral DNMTi. Complete Response (CR) was achieved in 22% (95% CI 15.1, 29.8), and overall response (CR + Partial Response + marrow CR + Hematologic Improvement) of 62% (95% CI 52.8, 69.9) was similar to seen with parenteral DNMTi. K-M estimated mOS was 31.7 months.

Conclusions: Oral decitabine/cedazuridine is the only DNMTi demonstrating equivalent pharmacokinetic exposure to its IV form, and led to expected equivalent responses, with mOS of 31.7mo in this study. Additional studies using oral decitabine/cedazuridine in combination with new oral agents for hematological disease are warranted.
DEVELOPMENT OF SECONDARY RESISTANCE TO HYPMETHYLATING AGENTS

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Background And Aims: Hypomethylating agents (HMAs) 5-azacytidine (AZA) and 5-aza-2'-deoxycytidine (DAC) are used in treatment of elderly patients with MDS and AML. However, many patients do not respond to the treatment and others eventually stop responding following the secondary resistance development. Aim of our study is to identify mechanisms of secondary resistance to HMA, which may finally lead to overcoming its development and to prolonging the response of patients.

Methods: In our laboratory, AZA- and DAC-resistant cell sublines were developed from two AML cell lines (MOLM-13 and SKM-1). Cytotoxicity of drugs in our cell models was determined using MTS assay. Expression of genes was analyzed by RT-PCR and Western blot.

Results: No important differences were observed in expression of genes involved in metabolism and transport of HMAs at mRNA level in AZA- and DAC-resistant sublines compared to parental cell lines. However, in DAC-resistant cell sublines down-regulation of deoxycytidine kinase (DCK), enzyme responsible for DAC activation, was observed at protein level. These sublines were also cross-resistant to other antineoplastic drugs dependent on activation by DCK, but not to AZA, which is not activated by this enzyme. While two out of three AZA-resistant cell sublines were also non-cross-resistant to second HMA, one of these sublines showed decreased sensitivity towards DAC.

Conclusions: Our results suggest that down-regulation of DCK may play important role in the secondary resistance towards DAC and that probably more than one mechanism of secondary resistance towards HMAs exist. This work was supported by grants from the Slovak APVV (APVV-19-0093) and VEGA grant agencies (VEGA 2/0057/18).
AZACYTIDINE-BORTEZOMIDE COMBINATION FOR SECONDARY MYELOID MALIGNANCIES. SINGLE CENTRE EXPERIENCE.

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**Background And Aims:** BACKGROUND: Azacytidine is an analogue of cytidine, and is indicated to patients with specific myeloid malignancies. Bortezomide is a proteasome inhibitor with the main indication of the multiple myeloma to which mantle lymphoma was added. Recently studies have been done on its combined use (V-V) in myeloid malignancies with positive results. AIM: The aim of the presentation is to present our clinical experience in the use of azacytidine-bortezomide in four cases with secondary myeloid malignancies.

**Methods:** METHODS: Four patients, three men and one woman, an average age of 68.5 years (62-84), were included. The regimen was given in 28-day cycles including azacytidine 75mg/m² days 1 to 7 and bortezomide 1.3mg/m² days 1,4,8,11.

**Results:** The following table showed the clinical data and characteristics of the included patients.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Initial Diagnosis</th>
<th>Date</th>
<th>Therapy</th>
<th>Second Diagnosis</th>
<th>Date</th>
<th>Therapy before V-V</th>
<th>V-V cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>Male</td>
<td>High-risk MDS</td>
<td>2014</td>
<td>None (cardiac operation)</td>
<td>sAML</td>
<td>2015</td>
<td>3+7</td>
</tr>
<tr>
<td>Patient 2</td>
<td>Male</td>
<td>Multiple Myeloma</td>
<td>2017</td>
<td>Myeloma protocols</td>
<td>sMDS, monosity 7</td>
<td>2020</td>
<td>None</td>
</tr>
<tr>
<td>Patient 3</td>
<td>Male</td>
<td>Amyloidosis</td>
<td>2012</td>
<td>Autologous transplantation</td>
<td>sMDS, complex Karyotype</td>
<td>2015</td>
<td>None</td>
</tr>
<tr>
<td>Patient 4</td>
<td>Female</td>
<td>Multiple Myeloma</td>
<td>2009</td>
<td>Autologous transplantation</td>
<td>MDS del20q/sAML</td>
<td>2015</td>
<td>3+7</td>
</tr>
</tbody>
</table>

**Conclusions:** CONCLUSION: It seems that the combination of azacytidine - bortezomide is effective, well tolerated offering a satisfactory quality of social and professional life. Large studies are needed to prove whether this combination can be a bridge to allogeneic transplant or benefit the non-transplantation candidates.
LENALIDOMIDE TREATMENT IS ASSOCIATED WITH LOWER SERUM LIPIDS

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Background And Aims: Two patients on Lenalidomide (Len) experienced reduced serum lipids. This stimulated a retrospective analysis. The aim was to assess the association of Len on a patient's lipid profile.

Methods: Eligible patients were on Len (2001-2018), for myeloma or MDS. They also had serum triglycerides (TG), total cholesterol (TC) and LDL-cholesterol (LDL-C), at baseline, during and following Len treatment. We analyzed patients with or without statins.

Results: In the TG, TC and LDL-C groups there were 94, 102 and 85 patients, respectively. The mean age was 70 years and F/M ratio 1:1. Almost half of them were on statins. The mean baseline serum TG level prior to Len treatment was 163.5 mg/dL. It decreased during treatment to 136.6 mg/dL (p<0.005), and increased after Len termination to 176.1 mg/dL (p<0.005). The difference between the baseline and post-Len TG levels was not statistically significant. The corresponding values of TC, and LDL-C, were 186.6, 161.8, 171.6 mg/dL and 101.7, 84.2, 93.6 mg/dL, respectively. In order to neutralize the effect of statins, each group was divided into statin-treated and untreated. All 3 lipids had the same pattern, with decreased serum levels during Len treatment, and a significant increase after Len discontinuation, close to the baseline levels.

Conclusions: Lenalidomide treatment is associated with reduced serum lipids in MDS and myeloma patients, with or without statins. Len discontinuation resulted in a serum lipid rise to baseline. Future research will further clarify the mechanism and the role of lenalidomide in lipid metabolism.
E-PERSON VIEWING

EFFICACY OF CPX-351 AS FIRST LINE TREATMENT FOR HIGHER RISK (HR) MDS/CMML. A STUDY BY THE GFM

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Background And Aims: Intensive chemotherapy (IC, 3+7) yields 40-50% CR in HR MDS but prolonged myelosuppression and 10-30% early deaths. It is also recommended before allo SCT when marrow blasts are increased. CPX-351, a liposomal combination of cytarabine (CYT) and daunorubicin (DNR), proved greater efficacy than classical 3+7 in secondary-AML, including after allo SCT. We evaluated response to CPX-351 in untreated HR MDS.

Methods: Between July 2020 and Jan 2021, 31 HMA and IC naïve HR MDS/CMML aged <70 years were included. CPX-351 induction was given at 44mg/m² (DNR)/ 100mg/m² (CYT)/ day on days 1, 3 and 5. Consolidation cycles (44mg/m² (DNR)/ 100mg/m² (CYT)/ one day) were planned (3 cycles). Allo SCT was permitted. The results from the first 20 patients included are presented.

Results: Median age was 64 years (48-68); WHO: 17 MDS-EB2, 3 CMML-2; IPSS: 17 int-2, 3 high; R-IPSS: 3 intermediate, 12 high, 5 very high; karyotype: 14 good,2 intermediate, 3 poor, 1 very poor. Response rates (ELN 2017) were: CR: 50%, CRi: 15%, MLFS: 25%, stable: 10%. All but 2 patients with marrow blasts >10%, reached <5% blasts after induction treatment. Median time to platelets >20G/L was: 17 (0-55) days and to ANC >1G/L: 30 (2-48) days. Only 2 patients had grade 3 mucositis. No patient died during induction treatment. Eleven patients have received allo SCT and 5 are planned for allo SCT.

Conclusions: CPX-351 is effective in HR MDS/CMML, especially for blast clearance, and as a bridge to allo SCT. Updated data will be presented.
PHASE 3 VERONA STUDY ASSESSING THE SAFETY AND EFFICACY OF VENETOCLAX WITH AZACITIDINE IN PATIENTS WITH NEWLY DIAGNOSED HIGHER-RISK MYELODYSPLASTIC SYNDROMES

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Background And Aims: Patients with higher-risk myelodysplastic syndrome (MDS) are typically treated with hypomethylating agents (HMAs) such as azacitidine. HMAs alone provide patients with higher-risk MDS limited improvement in clinical benefit. Venetoclax is an oral small molecule inhibitor of B-cell lymphoma 2 (BCL-2). In a Phase 1b study, venetoclax combined with azacitidine demonstrated favorable safety and efficacy in patients with newly diagnosed higher-risk MDS. The aim of this Phase 3 VERONA study (NCT04401748) is to evaluate the safety and efficacy of venetoclax combined with azacitidine in patients with newly diagnosed higher-risk MDS.

Methods:
Newly diagnosed patients per WHO 2016 classification of higher-risk MDS will be enrolled at ~200 sites globally (~500 patients). Patients must meet disease activity criteria including: IPSS-R score >3, ECOG ≤ 2, and no prior hematopoietic stem cell transplantation (HSCT). Patients will be randomized 1:1 to receive placebo or venetoclax 400 mg oral tablet once daily (Days 1-14) in combination with azacitidine 75 mg/m² (intravenous or subcutaneous) (7 days within the first 9 days per 28-day cycle). Patients will receive study treatment until disease progression, unacceptable toxicity, HSCT, withdrawal of consent, or discontinuation.

**Results:** The dual primary endpoints are complete remission and overall survival. Secondary outcomes include RBC and platelet transfusion independence, change in fatigue ((PROMIS)-fatigue SF 7a) and time to deterioration of physical functioning (EORTC QLQ-C30).

**Conclusions:** Enrollment is ongoing in 18 countries.
CLINICAL EFFECT OF HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION COMBINED WITH POST-TRANSPLANT CYCLOPHOSPHAMIDE FOR REFRACTORY CYTOPENIA IN CHILDHOOD

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Background And Aims: To investigate the efficacy and safety of allogeneic haploidentical stem cell transplantation (allo-HSCT) in treatment of childhood myelodysplastic syndromes (MDS) with modified post-transplant cyclophosphamide (PT/Cy).

Methods: From July 2016 to February 2020, 9 children diagnosed with MDS in our hospital were retrospectively analyzed.

Results: 9 children diagnosed with MDS in our hospital included 2 males and 7 females. The median age was 6.9 years old (range, 1-15 years), the median medical history of MDS was 2.8 years (range, 3 months -8 years). Among the 9 patients, they were diagnosed with RCC. The donors were from family members and HLA matching 6-9/12 loci were identical. The donors were all healthy after screening, and did not carry the pathogenic gene. The median age was 37.3 (25-49) years old. Graft-versus-host disease (GvHD) prophylactic regimen was started 3-4 days after transplantation, where 50mg/kg of cyclophosphamide was administered by intravenous infusion. From day 5 after transplantation, low-does tacrolimus was administered by intravenous infusion and mycophenolate mofetil was administered orally. Eight patients were successfully transplanted in the end, and one of them were graft failure. After the second transplantation, the transplantation was successful. All the patients achieved full donor chimerism after transplantation. The transplantation was successful after the second transplantation. All patients obtained complete donor cell chimerism after transplantation. All 9 patients were median followed up of 784 (range 423-1290) days. Both overall survival rate and disease-free survival rate were 100%.

Conclusions: Haploid hematopoietic stem cell transplantation combined with post-transplant cyclophosphamide is a safe and effective treatment for children with RCC.
OUTCOMES OF HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION OF PATIENTS FROM THE MDS LATIN AMERICAN REGISTRY


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Background And Aims: Haplo-Hematopoietic Stem Cell Transplantation is valuable alternative in the absence of an HLA-matched donor in patients with Myelodysplastic Syndrome. Aim: to evaluate the outcomes of haplo HSCT in patients from MDS Latin American Registry.

Methods: We analyzed data from 26 patients with MDS from the transplant registry of 19 centers in Latin America from 1989 to 2020.

Results: Mean age was 44.8 years with a predominance of males (75%). Regarding to the Prognosis Scoring System (IPSS-R), the majority of patients were high risk (37.5%). Myeloablative conditioning regimen was performed in 5 patients (19, 2%), non-myeloablative in 4 (15, 4%) and reduced intensity in 17 (65.4%). The cell sources were peripheral blood (PB) (n=15; 57.7%) and bone marrow (BM) (n= 11; 42.3%). Cytogenetic was performed in 21 patients (84.6%), with 8 (38.09% including very and good), 4 (19.05%) intermediate and 9 (42.86%) poor and very poor MDS stratification. Only 5 patients (19.2%) used hypomethilating before HSCT. The main post-HSCT complications were mucosite (65,38%), other infections (46,15%) and cytomegalovirus (26,92%). Overall survival was 78,84% in two years. There were significant differences for risk of death according to: age (≤50 vs >50- p=0,046; OR:0.07; 95%CI:
0.003663-1.540) and cell source (BM vs PB-p=0.007; O.R:26.23; 95%CI: 1.258-546.8). There was no difference for risk of acute and chronic GVHD regarding age and cell source. It was not observed differences regarding karyotype and death or GVHD.

**Conclusions:** Age ≥ 50 years and bone marrow as source of stem cell seem to influence the mortality in the studied population.
COMPARABLE OUTCOMES AMONG ADULT PATIENTS ALLOTRANSPLANTED FOR MYELODYSPLASTIC SYNDROME USING HAPLOIDENTICAL, MATCHED UNRELATED OR MATCHED SIBLING DONORS: A SINGLE-CENTER STUDY.

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Background And Aims: Allogeneic stem cell transplantation (allo-SCT) remains the only curative option for patients with myelodysplastic syndrome (MDS). If recent data have shown encouraging results with haploidentical (haplo) donors in this context, no comparison with allo-SCT using other source of graft (matched sibling [MSD] or unrelated [MUD] donors) has been reported so far.

Methods: We retrospectively considered 102 consecutive adults transplanted for MDS between March 2010 and August 2020 in our Department, comparing outcomes between those receiving a graft from a MSD (n=33), a MUD (n=48) or a haplo-donor (n=21).

Results: With a median follow-up of 46.4 months, the 4-year OS was comparable between the three groups (haplo: 60.1 % ± 11.0 % , MSD: 59.0 % ±9.4 % and MUD: 61.2 % ± 7.2 %, p = 0.88) as well as the 4-year DFS (55.9 % ± 11.1 % vs 51.2 % ±9.2 % vs 59.6 % ± 7.2 %, p = 0.78) and the cumulative incidence (CI) of NRM (34.6 % ±12.4 % , 15.4% ± 6.4% and 23.8 % ± 6.4 %, p = 0.21). Also, the 4-year CI of acute grade 3-4 GVHD (14.3% vs 15.2% vs 20.8%, p=0.79) and of moderate/severe chronic GVHD (14,3% vs 24.2% vs 27,1%, p=0.56) were not significantly different, translating in a similar 4-year CI of GRFS (56,1 % ± 11,0% vs 28,1% ±9,2 % vs 32,8 % ± 7,4%,p=0 .41).

Conclusions: A haplodonor represents a valid alternative in MDS patients lacking a MSD or a MUD for allo-SCT.
ALLOGENIC STEM CELL TRANSPLANT FOR FANCONI ANEMIA A SINGLE CENTER EXPERIENCE

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Background And Aims: Fanconi anemia (FA) is a rare heterogeneous disorder characterized by congenital malformations, progressive marrow failure, and predisposition to leukemia and epithelial malignancies. Allogeneic stem cell transplantation (alloHSCT) is the only therapy that can correct the hematological manifestations of FA patients.

Methods: Retrospective analysis of all FA patients, who underwent match family (MFD), unrelated cord blood (UCB) or haploidentical allogeneic HSCT at King Hussein Cancer Center (KHCC) from January, 2005 until December, 2019, median follow up of 38 months.

Results: Thirty patients underwent alloHSCT, the median age was 8.5 years. Twenty-four donors were MFD, three patient underwent UCD transplant, and 3 underwent T-cell repleted haploidentical transplant. All MFD and UCB patients received fludarabine based conditioning, single fraction TBI was added to 3 patients with Myelodysplastic syndrome (MDS). Haploidentical transplant patients received Fludarabine and single fraction TBI, post-transplant Cyclophosphamide was used for T-cell depletion. Twenty-four patients developed CMV reactivation, seven patients developed acute GVHD, and eleven patients developed chronic GVHD. Four patients underwent second alloHSCT, three due to primary graft failure, and one developed donor type aplasia 2 years post-transplant. Two patients developed metastatic squamous cell carcinoma, both died 5.5 and 12 years post alloHSCT, none has received irradiation. We report another 3 deaths, one due to chemotherapy toxicity before engraftment, one on day 31 post-transplant due to pulmonary and intracranial hemorrhage and the last died one year post-transplant due to encephalopathy, three out of 5 deaths were in MDS patients.

Conclusions: Bone marrow stem cells from MFD is preferred for alloHSCT in fanconi anemia. Extended family search should be done identify potential donors. FA patients with MDS have lower survival post-transplant as reported in literature.
POST-TRANSPLANTATION CYCLOPHOSPHAMIDE FOR GRAFT-VERSUS-HOST DISEASE PROPHYLAXIS IN ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION FOR HIGHER-RISK MYELODYSPLASTIC SYNDROME

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Background And Aims: Allogeneic HCT is an only potentially curative option for patients with higher-risk MDS. In this study, we aimed to evaluate the feasibility of post-transplantation cyclophosphamide (PTCy) as GVHD prophylaxis in allogeneic HCT for higher-risk MDS. We compared outcomes of PTCy and those of historical group using anti-thymocyte globulin (ATG).

Methods: Patients with higher-risk MDS or MDS/MPN were included. Higher-risk MDS was defined by MDS with IPSS >1.0 or BM blast ≥ 5% at any time points before HCT. The conditioning regimen consists of busulfan (4-days for <55 years, 2-days for ≥55 years) and fludarabine. For GVHD prophylaxis, PTCy (50 mg/kg on D3-4), cyclosporine, and MMF were administered. In ATG group, 2- or 4-days of busulfan, fludarabine, and ATG with MTX and cyclosporine were used.

Results: Ninety-two and 144 patients received allogeneic HCT using PTCy and ATG, respectively. The median overall survival was 47.9 and 44.0 months in each group (P=.383). Cumulative incidence of total and grade II-IV acute GVHD in PTCy and ATG group were 19.6% vs. 37.5% (P=.002), and 2.6% vs. 21.7% (P<.001), respectively. Total and extensive chronic GVHD (50.0% vs. 49.1%, P=.567; 32.5% vs. 33.4%, P=.581), 1-year NRM (20.8% vs. 22.9%, P=.702), and 2-year relapse incidences (16.0% vs. 18.1%, P=.605) were not different between two groups. Engraftment was significantly faster with ATG than PTCy (median 12 vs. 15 for neutrophil; 15 vs. 24 days for platelet).

Conclusions: Allogeneic HCT using PTCy as GVHD prophylaxis in higher-risk MDS seems feasible in terms of low acute GVHD and relapse incidence.
E-POSTER VIEWING

MDS IRON ROAD: AN INTERNET-BASED ALGORITHM FOR THE DIAGNOSIS, WORKUP AND MANAGEMENT OF IRON OVERLOAD IN MDS FROM THE CANADIAN CONSORTIUM ON MDS (CCMDS)

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Background And Aims: Transfusional IOL is detrimental to clinical endpoints in MDS. A comprehensive internet-based algorithm on IOL, the MDS Iron Road was developed by the CCMDS.

Methods: Reviewed were mechanisms of IOL induced cellular damage, evidence for clinical endpoints impacted: organ dysfunction, infections, marrow failure, overall survival, AML progression, and endpoints around hematopoietic stem-cell transplant. Evidence for impact of IOL reduction is discussed, decision points identified, areas identified where evidence is suboptimal and practical recommendations made. Evidence levels and grading of recommendations are provided.

Results: The Iron Road gives an approach to management of IOL in MDS in clinical practice. Reference to the MDS Clear Path (http://www.MDSClearPath.org) gives information on MDS management with details of treatment options. The Iron Road provides in-depth discussion of transfusions, IOL, iron reduction strategies, ICT agents available, dosing and administration, monitoring, dose adjustments, expected response, and side effects and their management. The “IR Express” is a series of questions specific to the patient’s clinical status which results in a treatment recommendation; the self-directed mode (Figure 1) presents the overall algorithm with links to details. Information panels discuss supporting evidence. References with links to abstracts and supportive data are included. The Iron Road is available by entering https://mdsironroad.org into a browser; username ironroad, password
Conclusions: An internet-based algorithm is available to support management of IOL in MDS and should help standardize IOL care. Content will be updated to reflect advances made in IOL care in MDS.
COMPARATIVE STUDY ON IRON CONTENT DETECTION BY ENERGY SPECTRAL CT AND MRI IN MDS PATIENTS

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Background And Aims: The purpose of this study was to identify the difference between dual energy spectral computed tomography (DECT) and 3.0T magnetic resonance imaging (MRI) used to detect liver/cardiac iron content in Myelodysplastic syndrome (MDS) patients with different adjust serum ferritin (ASF) levels.

Methods: Liver and cardiac iron content were detected by DECT and MRI. Patients were divided into different subgroups according to the level of ASF. The receiver operating characteristic curve (ROC) analysis was applied in each subgroup. The correlation between iron content detected by DECT/MRI and ASF was analyzed in each subgroup.

Results: ROC curves showed liver virtual iron content( LVIC) Az was significantly less than liver iron concentration (LIC) Az in the subgroup with ASF<1000 ng/ml, but higher than LIC Az in the subgroup with5000≤ASF ng/ml. In patients undergoing DECT and MRI examination on the same day, ASF was significantly correlated with LVIC but not with LIC. After removing the data of ASF > 5000 mg/L in LIC, LIC became correlated with ASF. Larger sample shows LIC was not correlated with ASF in patients with 2500ng/mls ASF. Neither cardiac VIC nor myocardial iron content (MIC) were correlated with ASF in these
Fig. 1 (a) ROC curves showed the comparison of liver VIC (LVIC) and LIC in differentiating patients with ASF <500 ng/ml or 500 <ASF <1000 ng/ml (9 patients). LIC A2 was 0.889, and LIC at 1.8 (mg Fe/g) sensitivity and specificity were 100% and 33.3%, respectively. LVIC A2 was 0.694, and LVIC at 1.34 (mg/ml) sensitivity and specificity were 33.3% and 66.7%, respectively. There was significant difference between LVIC and LIC sequences (p < 0.05). (b) ROC curves showed the comparison of LVIC and LIC in differentiating patients with ASF <1000 ng/ml or 1000 <ASF <2500 ng/ml (17 patients). LIC A2 was 0.903, and LIC at 3.2 (mg Fe/g) sensitivity and specificity were 100% and 33.3%, respectively. LVIC A2 was 0.819, and LVIC at 1.85 (mg/ml) sensitivity and specificity were 62.5% and 88.9%, respectively. There was no significant difference between LVIC and LIC sequences (p > 0.05). (c) ROC curves showed the comparison of LVIC and LIC in differentiating patients with ASF <2500 ng/ml or 2500 <ASF <5000 ng/ml (20 patients). LIC A2 was 0.750, and LIC at 7.0 (mg Fe/g) sensitivity and specificity were 75% and 56.2%, respectively. LVIC A2 was 0.875, and LVIC at 2.69 (mg/ml) sensitivity and specificity were 75% and 81.2%, respectively. There was no significant difference between LVIC and LIC sequences (p > 0.05). (d) ROC curves showed the comparison of LVIC and LIC in differentiating patients with ASF <5000 ng/ml or 5000 <ASF ng/ml (23 patients). LIC A2 was 0.617, and LIC at 15.0 (mg Fe/g) sensitivity and specificity were 100% and 30%, respectively. LVIC A2 was 0.883, and LVIC at 4.03 (mg/ml) sensitivity and specificity were 100% and 85%, respectively. There was significant difference between LVIC and LIC sequences (p < 0.05).
Fig. 2 Underwent DECT and MRI examination on the same day: (a) Liver VIC was correlated with ASF ($r=0.65$, $p<0.05$) (b) LIC was not correlated with ASF ($r=0.11$, $p>0.05$). (c) LIC was correlated with ASF after the exclusion of three ASF >5000ng/ml corresponding data ($r=0.55$, $p<0.05$).
**Conclusions:** In MDS patients with high iron content, such as ASF ≥5000ng / ml, DECT was more reliable than MRI in the assessment of iron content. But in low iron content, such as ASF < 1000 ng / ml, MRI is more reliable than DECT. Therefore, for sake of evaluating the iron content more accurately, the appropriate detection method can be selected according to ASF.

**Fig.3 Underwent DECT/MRI examination:** (a1) There were significant correlation between Liver VIC and ASF in the subgroup with ASF<1000ng/ml ($r=0.691$, $p<0.05$). (a2) There were significant correlation between Liver VIC and ASF in the subgroup with 1000≤ASF<2500ng/ml subgroup($r=0.378$, $p<0.05$). (a3) There were significant correlation between Liver VIC and ASF in the subgroup with 2500 ng/ml≤ASF ($r=0.723$, $p<0.05$). (b1) There were significant correlation between LIC and ASF in the subgroup with ASF <1000ng/ml ($r=0.38$, $p<0.05$). (b2) There were significant correlation between LIC and ASF in the subgroup with 1000 ≤ASF <2500ng/ml ($r=0.25$, $p<0.05$). (b3) There were no significant correlation between LIC and ASF in the subgroup with 2500 ng/ml≤ASF subgroup($r=0.03$, $p>0.05$).
MONITORING OF FERRITIN LEVELS AFTER BLOOD TRANSFUSIONS IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Background And Aims: In patients with myelodysplastic syndromes (MDS) with >20 blood transfusions and plasma ferritin levels >1000 µg/L, (inter)national guidelines indicate iron chelation therapy (ICT) to reduce potential organ damage due to transfusion-mediated iron overload. The aim of this study was to determine to what extent plasma ferritin levels were monitored in daily practice, and which factors contributed to monitoring plasma ferritin levels during the transfusion period.

Methods: We performed an observational, population-based study, using the Hemobase Registry, in MDS patients diagnosed between 2005 and 2017 in Friesland, a Dutch province with approximately 650,000 inhabitants. Clinical information on blood transfusions, plasma ferritin measurements and ICT was collected from electronic health records.

Results: A total of 237 out of 292 MDS patients (81.1%) received at least one transfusion. During the first 20 transfusions, plasma ferritin measurements were present in 115 of 237 patients (48.5%). After >20 transfusions, plasma ferritin measurements were present in 50 of 121 patients (41.3%). Age, comorbidities and IPSS-R score were not significantly associated with the likelihood of monitoring plasma ferritin levels. ICT was given to 22.3% (n=25) of patients eligible for ICT. Plasma ferritin levels were monitored in 65.0% of patients during
Conclusions: In this population-based study, plasma ferritin levels were measured in <50% of MDS patients with transfusions. ICT was not given to all eligible patients and monitoring of plasma ferritin levels during ICT was not performed in all cases. Plasma ferritin levels should be monitored more often to optimize decision-making and ensure appropriate patient care.
RED BLOOD CELL ALLOIMMUNIZATION AND AUTOIMMUNIZATION IN TRANSFUSION-DEPENDENT MDS AND MDS/MPN

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Background And Aims: Red blood cell (RBC) alloimmunization is a common complication in chronically transfused patients with MDS and MDS/MPN. There is no consensus to date on a transfusion strategy to prevent alloimmunization in these patients. The aims of this study were to determine the prevalence and characteristics of allo/autoimmunization in MDS and MDS/MPN transfusion-dependent (TD) patients, and to assess their impact on RBC requirements.

Methods: TD patients with MDS or MDS/MPN receiving follow-up in our hospital between January’19-March’21 were selected (N = 53). Our present transfusion policy consists in Rh/Kell matching from the first alloimmunization. RBC requirements in the 3 months prior and post-alloimmunization were registered.

Results: 32% of patients (n=17) developed alloantibodies, 11/17 with multiple alloimmunization. 31 alloantibodies were registered in total, 64.5% of them against Rhesus and Kell antigens. The median number of RBC units until alloimmunization was 12.5, with a median time to alloantibody detection from the start of TD of 5 months. Alloimmunized patients more frequently had low risk MDS (58.8% vs. 33.3%) and were female (47.1% vs. 22.2%). The prevalence of autoantibodies was significantly higher in alloimmunized patients (29.4% vs. 0%; \( P=0.002 \)). Alloimmunization was followed by an increase in RBC requirement in 77% of patients: 3(0-18) vs. 6(0-26); \( P=0.05 \).

Conclusions: The prevalence of alloantibodies in our cohort supports the need to change transfusion policies in this group of patients in order to prevent alloimmunization from the start of transfusion dependence.
Invited Speakers Abstracts

PLENARY SESSION: CLINICAL RESEARCH: TARGETED AGENTS, PROGNOSIS AND PREDICTIVE MODELS
09/25/2021  12:30 PM - 02:10 PM

Presentation Name: How machine learning can contribute to prognostic and predictive models

HOW MACHINE LEARNING CAN CONTRIBUTE TO PROGNOSTIC AND PREDICTIVE MODELS

Aziz Nazha
Amazon Web Services

Machine learning is a branch of computer science that generates predictive or descriptive models by identifying patterns and connections in training data rather than by being explicitly programmed. The application of machine learning and artificial intelligence in medicine has gained substantial interest, both as a catalyst for research and as a means of improving clinical care from diagnosis to prognosis, and treatment selections for diseases. These applications include the management of haematological malignancy, in which machine learning has created inroads in pathology, radiology, genomics, and the analysis of electronic health record data. As our data continues to increase exponentially, the computational power to analyze this data becomes cheaper, and the tools to implement these technologies become more democratized, it is likely that these technologies become part of our day-to-day practice. Understanding the strengths and the weaknesses of these algorithms and how to appropriately integrate them in our clinical and research practices become very important. In this session, we will review how machine learning and artificial intelligence can be used in hematology to improve the diagnosis, prognosis, and selection of therapy in patients with Myelodysplastic Syndromes. We will also discuss current challenges and opportunities in applying these technologies in MDS and other myeloid malignancies.
Disease progression to AML occurs in ~30% of MDS patients. The identification of dysregulated genes/pathways has led to a better understanding of MDS pathophysiology and has identified new therapeutic targets (Pellagatti et al., Blood 2018). The utility of aberrant gene expression in the prediction of MDS patient survival and transformation to AML has been demonstrated (Gerstung et al., Nat Commun 2015). Recent studies using patient cells and cellular models have identified key differentially expressed genes and dysregulated pathways associated with MDS disease progression.

The p53 regulator MDMX is overexpressed in a high proportion of AML cases, and a recent study by Ueda et al. (Cancer Cell 2021) showed a higher transformation rate to AML in MDS patients with high MDMX expression. Mechanistically, MDMX binds to CK1α and prevents CK1α-dependent degradation of β-Catenin, and MDMX overexpression leads to clonal expansion and leukemic transformation. Importantly, this study demonstrated that the MDMX/CK1α/β-Catenin axis is therapeutically targetable in myeloid leukemia.

The combined power of induced pluripotent stem cells (iPSCs) and CRISPR/Cas9 technology has been harnessed for the modeling and study of disease progression in myeloid malignancies. A recent study by Wang et al. (Cell Stem Cell 2021) showed that the sequential introduction of ASXL1, SRSF2, NRAS and FLT3 mutations in iPSCs tracked clonal evolution to AML, with transcriptional programs driving specific transitions between disease stages.

Emerging data show that some of the downstream effects of different mutated splicing factors converge on common cellular pathways/processes. SF3B1, SRSF2 and U2AF1 mutations have been shown to enhance NF-κB signaling via aberrant splicing of distinct target genes (Smith et al., Nat Cell Biol 2019; Lee et al., Cancer Cell 2018). U2AF1 mutations induce oncogenic isoforms of IRAK4 in myeloid malignancies that are therapeutically targetable.

The study of the MDS transcriptome has provided deep insights into disease pathogenesis.
Allogeneic stem cell transplantation (alloSCT) is the only curative therapy for MDS. However, success following alloSCT is limited by relapse and treatment-related morbidity and mortality (TRM). Given that alloSCT should routinely be recommended for patients with higher-risk MDS, several strategies are currently being tested to improve outcomes, with a focus on relapse reduction, minimization of TRM, or a combination of both. Understanding the correct conditioning regimen is a critical component of NRM reduction. First, a correct assessment of comorbidity using the age-adjusted HCT-CI must be considered so that excess conditioning intensity is not delivered when not necessary and deemed too risky. Beyond measurable comorbidity is the transplant recipient’s capacity to heal from conditioning related injury, which may in turn be influenced by tissue healing capacity and by intrinsic telomere repair capabilities. An assessment of disease risk, most notably molecular mutation status should be considered, as certain mutations portend a poor prognosis in the context of myeloablation, and others derive no benefit from enhanced conditioning intensity. While some conditioning regimens are associated with improvement in TRM, others might be associated with less relapse following alloSCT. Devising myeloid-specific conditioning and incorporating agents with known activity against MDS are current priorities in the field. In addition to myeloid specific conditioning, strategies that reduce relapse following transplantation include the use of targeted agents given as maintenance as well as strategies to manipulate the donor immune system to its graft-vs-tumor effect. Finally, comprehensive care of the older MDS to maximize outcomes following alloSCT need to be employed. These strategies will be all reviewed during this session.
The clinical phenotype of myelodysplastic syndromes (MDS) is hallmarked by bone marrow dysplasia, cytopenias and propensity of transformation to acute myeloid leukemia. Of critical importance, comprehensive molecular analysis by next-generation sequencing (NGS) can identify somatic mutations in most patients having both prognostic and therapeutic implications. However, how these diverse classes of mutations can present with a common phenotype has been unknown. Importantly, understanding the key pathogenic drivers of MDS, particularly in lower risk MDS, have been recently advanced. In addition to aberrant innate immune signaling and a pro-inflammatory microenvironment, a central inflammatory process known as pyroptosis, or inflammatory cell death, via activation of the NLRP3 inflammasome and ultimately elaboration of inflammatory cytokines such as IL-1β, is critical in the pathogenesis of MDS. Notably, the danger-associated molecular pattern (DAMP) protein S100A9, with overexpression partially driven by somatic mutations, can trigger activation of the inflammasome while also expanding myeloid derived suppressor cells (MDSCs) leading to a feed-forward cycle to amplify pyroptotic cell death. Additionally, identification of components of the inflammasome complex, i.e. the ASC speck, can be a diagnostic biomarker for MDS. Most importantly, although therapies to date for lower risk MDS have been solely focused on cytopenia improvement (particularly erythropoiesis), novel biologically rational therapeutic strategies have begun to target these aberrant inflammatory signaling pathways (e.g. inhibitors of NLRP3, IL-1β, and IRAK4) with the goal of true disease modification. Additionally, activating both innate and adaptive immune responses, as is being evaluated with therapies targeting CD47 and TIM-3, has shown synergistic activity in combination with azacitidine in higher risk MDS patients but may also play a critical role to improve long term outcomes in lower risk MDS patients. Ultimately, combination trials may be required to change the natural history of MDS patients with significant optimism on the future treatment landscape.
Allogeneic hematopoietic cell transplantation (HCT) is the only curative treatment option for myelodysplastic syndromes and approximately 1000 such transplants are performed each year in the US. The presence of a “cure fraction” after allogeneic HCT along with other observations provide strong but indirect evidence that MDS is an immune-responsive disease. Nonetheless, relapse remains the leading cause of death after HCT for MDS, and clinical prediction models for post-HCT outcomes implicate blast percentage at transplantation and cytogenetic or molecular abnormalities as predictive of relapse. Why and how MDS-associated mutations interact with allogeneic T cells remains unknown.

In MDS, it is particularly difficult to differentiate residual malignant cells from their normal counterparts since they have similar morphology and immunophenotype. Recent developments in single cell sequencing allow the detection of copy number variations (CNV), single nucleotide variants (SNV), and surface protein expression in individual cells, finally allowing the possibility to trace a genotype-phenotype relationship between immune and malignant sub-populations in the marrow. If the interactions between immune cells are a conversation, the words and sentences of this conversation are receptors, ligands, cytokines, enzymes and signaling pathways. The vocabulary of interaction between MDS cells and immune cells is the MDS “immunome”.

One of the difficulties in treating myeloid malignancies with CART cells has been the lack of clearly-defined, safe antigens to target. That is because all myeloid-associated antigens are also present on at least some early hematopoietic and/or myeloid cells, making it challenging to envision a scenario akin to that of successful B cell directed CART cell therapy, wherein long-term lineage aplasia correlates with CART cell function and relapse-free survival. One potential approach to circumvent this issue is by engineering a leukemia-specific antigen. This has been done preclinically by our group and others using CRISPR/Cas9 to delete a shared antigen (CD33) from normal hematopoietic stem/progenitor cells (HSPC), followed by anti-CD33 CART cells that ablate all CD33-expressing cells (AML or MDS and residual recipient hematopoiesis) while leaving the CD33-deficient, engineered donor stem cells untouched. Nonetheless, CART cell therapy for myeloid malignancies is feasible even without the gene-engineered HSPC platform, provided the patient can be supported through a period of CART-mediated bone marrow aplasia.
Red blood cell (RBC) transfusion dependence (TD) in MDS portends inferior clinical outcomes. A priority objective of MDS therapy is to achieve hematologic improvement (HI), particularly in the erythroid lineage, to reduce RBC transfusion requirements, improve quality of life, and alter MDS natural history. Iron accumulates in TD patients resulting in iron overload (IOL). Excess iron can affect tissues and organs and through the ability of iron to transfer electrons, result in oxidative stress, which alters lipids, proteins and nucleic acids, resulting in cellular consequences. IOL is responsible for some of the adverse outcomes associated with TD but it remains to be defined what IOL level, by which measure, is most relevant to particular outcomes. Here measurements of IOL in clinical practice are reviewed (transfusion iron burden, serum ferritin level, organ iron, oxidative stress), with discussion of clinical endpoints examined (OS, leukemia free survival, organ endpoints [cardiac, hepatic, endocrine], suppression of hematopoiesis, data in higher risk MDS and around hematopoietic stem cell transplantation]). Future investigations should aim to standardize IOL severity, and prospectively verify targets of successful iron reduction. A classification of IOL severity is proposed, as are response criteria for IOL reduction. Iron reduction should be undertaken wherever possible using medications that induce HI with or without phlebotomy, and otherwise iron chelation should be considered, recognizing that dose of chelator may be limited by tolerance. An on-line algorithm detailing the workup, diagnosis and management of IOL in MDS, and ways to reduce IOL, with supporting information included, will be highlighted. The emerging importance of iron induced oxidative stress will be discussed, including transferrin saturation, recently shown to be associated with clinical endpoints. In conclusion, multiple data indicate the importance of IOL management and reduction in optimizing clinical outcomes in MDS, and further refinement of successful iron reduction targets is warranted.
Inherited predisposition to myeloid malignancies is due to deleterious germline variants in an increasing number of genes. Recognition of a germline deleterious variant has important health implications for the management of the index patient and his/her family members. In my talk, I will outline strategies and challenges in diagnosis of these disorders; what we know about the natural history of some of these disorders; and guidelines for patient management. Germline predisposition to myeloid malignancies is more common than historically appreciated, and with greater numbers of genes and mutation types identified, the complexity of clinical testing also increases, challenging busy practitioners to diagnose patients accurately. Recognition of germline cancer predisposition also complicates planning for allogeneic stem cell transplantation in which relatives are often the preferred donors. I will outline the numerous challenges to diagnosis of these conditions. Key features of the initial clinical presentation that signal a likelihood of having germline predisposition include: personal history of two or more cancers; personal history of a hematopoietic malignancy along with a family history of: another hematopoietic malignancy/prolonged cytopenia/or other hematologic abnormality such as macrocytosis or onset of a non-hematopoietic tumor at an age < 50 years old (yo) within two generations of the proband; and/or molecular testing of hematopoietic cells showing a deleterious variant in a gene known to confer a hereditary hematopoietic malignancy (HHM), especially one that persists despite change in disease status (i.e., persists from diagnosis through remission). As we identify more germline predisposition genes and individuals with deleterious variants in those genes, we need to develop comprehensive treatment plans that include accommodations for the impact of these mutations on the function of hematopoietic stem cells and other organ function. We also need to provide supportive genetic counseling and clinical expertise to allow our patients to live the healthiest lives possible.
Signaling by the TGF-β superfamily is important in the regulation of hematopoiesis and is dysregulated in myelodysplastic syndromes (MDSs), contributing to ineffective hematopoiesis and clinical cytopenias. TGF-β, activins, and growth differentiation factors exert inhibitory effects on red cell formation by activating canonical SMAD2/3 pathway signaling. In the talk, we summarize evidence that overactivation of SMAD2/3 signaling pathways in MDSs causes anemia due to impaired erythroid maturation. We will also describe the basis for biological activity of activin receptor ligand traps, novel fusion proteins such as luspatercept that are promising as erythroid maturation agents to alleviate anemia and related comorbidities in MDSs and other conditions characterized by impaired erythroid maturation.
Myelodysplastic syndromes (MDS) mainly affect the elderly population which implicates that the majority of patients cannot tolerate intensive therapeutic approaches including allogeneic hematopoietic stem cell transplantation (allo-HSCT). Current standard of care approaches for lower-risk MDS patients comprise ESA, luspatercept for MDS-RS and iron chelation while HMA are not widely used. There is a tremendous unmet need for new treatments for MDS; rates of relapse are high and many patients are not eligible for existing approved therapies. Patients should therefore be offered clinical trial options across all disease stages. While many studies are evaluating agents with different mechanisms of action, most are still in early stages of development. The diverse nature of genetic mutations that drive MDS and other myeloid disorders, ranging from clonal hematopoiesis of indeterminate potential to AML, means that therapies need to be developed for specific patient subsets.

There are multiple novel approaches currently being examined in patients with low-risk MDS. Most of these agents aim to manage anemia and other cytopenias. Roxadustat is a hypoxia-inducible factor prolyl hydroxylase inhibitor that is being investigated in a phase 3 study for anemia in patients with low-risk MDS and low RBC transfusion burden. Imetelstat, a telomerase inhibitor, is currently in phase 2/3 study in RBC transfusion–dependent and ESA-relapsed or refractory low-risk MDS patients, with encouraging preliminary results. Finally, thrombopoietin-receptor agonists, such as romiplostim or eltrombopag, are not formally approved for patients with MDS but may be a treatment option for thrombocytopenia in patients with blasts <5%.
Patients with myelodysplastic syndromes (MDS) are classified as having higher-risk disease when they fall into advanced risk categories of the International Prognostic Scoring System (IPSS, score ≥1.5) or of the Revised IPSS (score >3.5). Standard initial treatment approaches for such patients includes one of the hypomethylating agents (azacitidine, decitabine, or decitabine/cedazuridine). Patients whose MDS includes certain molecular abnormalities that fall along methylation pathways (e.g., TET2) may be more likely to respond to HMAs, though don’t necessarily live longer, while those with additional abnormalities (e.g., ASXL1) may be less likely to respond. More recently, machine learning/artificial intelligence statistical approaches have been able to identify patients with discrete combinations of three molecular mutations who are more or less likely to respond to HMAs, and personalized predictive models that incorporate easy-to-obtain clinical variables to predict early in an HMA treatment course whether or not patients will respond to HMAs. Critical to maximizing response likelihoods is maintaining HMA treatment for a minimum of six treatment cycles, either by adjusting route of administration, setting expectations for treatment side effects, or educating patients and health care providers about the association between treatment duration and treatment response.
PLENARY SESSION: CLINICAL RESEARCH: TARGETED AGENTS, PROGNOSIS AND PREDICTIVE MODELS
09/25/2021 12:30 PM - 02:10 PM

Presentation name: What we learn from real-world registries

LESSONS FROM THE LONGTERM EXPERIENCE OF THE EUROPEAN MDS REGISTRY

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MDS and especially lower-risk MDS occurs mainly in persons older than 70 years who also suffer from chronic co-morbidities and reduced vitality. This leads to a lack of data from prospective randomized interventional studies for MDS.

The EUMDS Registry is an observational study in 17 countries, collecting longitudinal data of more than 2,800 unselected, newly diagnosed MDS patients with a follow-up of up to 13 years. The EUMDS Registry explored longitudinally new prognostic indicators, other than those integrated in the IPSS-R, such as red blood cell transfusion (RBCT) dependency, RBCT dose density (a better definition of RBCT intensity), health-related Quality of Life, platelet decline over time, labile plasma iron (LPI) and other iron toxicity associated parameters.

The RBCT dose density was associated with decreased PFS (P<0.001), even at very low dose densities (<0.75 unit/month). Clinically meaningful restrictions in the five dimensions of the EQ-5D index were more often observed in older patients and in those with more co-morbidities, lower Hb or RBCT dependency. A relative drop in platelets >25% at the landmark of 6 months predicted shorter 5-year survival. Iron toxicity was evaluated prospectively with several relevant markers of the iron metabolism, including LPI levels, over time. A multivariate analysis comparing elevated LPI levels and RBCT dependency to the control group with undetectable LPI and no RBCT need showed significantly decreased survival in all 3 risk groups (RBCT dependent and/or elevated LPI levels). Current interventions, such as ESA treatment, RBCT and iron chelation have been analyzed to prove the value of the new prognostic indicators.

Long term follow up of this LR-MDS population allowed us to demonstrate new poor prognostic indicators. This data show the relevance of carefully collected prospective, observational data in a disease of patients with advanced age who are usually not participating in randomized clinical trials.
Presentation name: *The Lower-risk Patient but higher-risk Disease*

**THE LOWER-RISK PATIENT BUT HIGHER-RISK DISEASE**

Prof. Moshe Mittelman  
Tel Aviv Sourasky (Ichilov) Medical Center, Tel Aviv University, Israel

Occasionally we see a patient who is classified, per IPSS or IPSS-R, with lower-risk MDS, but the course of the disease and the outcomes are more like a higher-risk disease. Can we use additional tools to better predict the prognosis of such "Lower-risk" MDS patients?

MDS prognostic factors can be categorized into 4 groups: 1) Well known established widely used factors, such as in the IPSS/R (Blast %, cytogenetics, CBC). 2) Reported but not widely used (age, comorbidities, fatigue, LDH, BM fibrosis). 3) Suggested potential but not studied yet with regard to prognosis (inflammation, micro-environment). 4) Factors that are unknown yet, but future digital tools will very likely detect.

We discuss the role of genetic signatures, including the expected IWG-PM model that is being developed, including the difficulties associated with the incorporation of mutations into the prognostic models.

Finally, we predict that future classifications will use digital tools that would allow incorporation of all available parameters and will result in a more accurate prognostic model.
PLENARY SESSION: CMML AND THE SPLICEOSOME

Presentation name: The cutting edge of the spliceosome

SPLICEOSOME GENE MUTATIONS IN MYELOID MALIGNANCIES

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Spliceosome genes are recurrently mutated in myeloid malignancies. The distribution of these mutations, as well as the unique hotspot mutations within SF3B1 and U2AF1 genes, varies across myeloid disease subtypes, suggesting that specific hotspot mutations induce different hematopoietic phenotypes. Furthermore, the two common U2AF1 mutants, S34F and Q157R, alter the splicing of two distinct sets of pre-mRNA targets and each co-occurs with unique gene mutations in MDS patients, suggesting these mutants may affect disease pathogenesis differently. Our results show that in mice, U2AF1S34F and U2AF1Q157R expression leads to different hematopoietic phenotypes. As expected, we observed altered consensus 3’ splice sites at the −3 position (for U2AF1S34F) and +1 position (for U2AF1Q157R) of differentially spliced exons, indicating altered but different pre-mRNA splicing induced by either U2AF1 mutant. Collectively, the results indicate that expression of two common U2AF1 mutants, Q157R and S34F, are not equivalent in vivo, potentially providing an explanation for their enrichment in different myeloid disease subtypes. While spliceosome gene mutations induce unique cellular phenotypes, they also induce shared phenotypes. For example, multiple spliceosome gene mutations induce R-loops, a three-stranded nucleic acid structure composed of a DNA:RNA hybrid and single-strand DNA, potentially creating a vulnerability that could be exploited therapeutically. Previous studies reported that inhibiting ATR, a critical mediator for R-loop resolution, also increases R-loops in cells and reduces spliceosome mutant cell viability compared to wild-type treated cells. We observe that inhibition of nonsense-mediated RNA decay (NMD) also induces R-loops and reduces spliceosome mutant cell viability. The reduced cell viability is R-loop dependent, further supporting a possibility that pharmacologic induction of R-loops may be a viable therapeutic approach to target spliceosome mutant cells.
Approximately one-third of patients diagnosed with MDS will have their disease progress to Acute Myeloid Leukemia. The likelihood of this happening varies depending on the MDS subtype and prognostic score in addition to other contributors such as, immune dysfunction and progressive genetic alterations. Leukemia arising from underlying MDS is classified as secondary AML. Using an illustrative case study, this presentation will review the clinical manifestations, bone marrow findings, prognosis and management strategies for patients with secondary AML.
A MAP TO SUPPORT PATIENTS AND CAREGIVERS ON THE MYELODYSPLASTIC SYNDROMES JOURNEY

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Moffitt Cancer Center - Department of Malignant Hematology

Background: Patients diagnosed with myelodysplastic syndromes and their caregivers encounter many obstacles in their cancer path.

Objectives: 1) To increase awareness of current challenges for MDS (Myelodysplastic syndromes) patients and caregivers 2) Provide guidance for support 3) Engage MDS nurse leadership board in review of recent literature on unmet needs of MDS patients and their caregivers.

Methods: A review of the literature was performed in Google Scholar, PubMed, and Cumulative Index of Nursing and Allied Health Literature to include publications from 2017-2021 that included the terms unmet needs in myelodysplastic syndromes. All publications were reviewed to identify themes for the unmet needs of patients and caregivers with MDS.

Results: Ten manuscripts met the inclusion criteria. Unmet needs for MDS patients from these reviews included the following categories: informational, emotional, physical, daily living/practical, and family life/relational. Fatigue was reported as the most common physical symptom that caused distress. The five most frequent symptoms contributing to distress were fatigue, pain, worry, sleep, and tingling in the hands or feet. A consistent theme from the review was the need for accurate information and discussion about MDS from the healthcare team. A need was also identified for palliative care research in MDS.

Conclusions: The map for supporting MDS patients starts with acknowledging that caregivers are critical for a successful journey. The second direction on the journey is providing detailed information on MDS focused on the individual, and their needs. Further research is necessary to improve management of fatigue and evaluate other symptoms systematically to be optimally managed by the healthcare team.

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Background: Myelodysplasia diagnosis widely varies in disease course and prognosis based on the type of MDS and the risk category.

Objectives: To review key patient and disease characteristics that impact treatment decisions for patients with high-risk myelodysplasia. Recognize management considerations of adverse events associated with treatment regimens. Apply the above principles to clinical case scenario.

Methods: A review of the literature was performed in CINHAL, Ovid, and Pub med, to include publication from 2010 to 2021 that include high risk myelodysplasia differentiation and treatment options. Case study; application of the data.

Results: Internationally approved diagnostic/prognostic criteria were reviewed for high-risk MDS. Standardization of treatment options were reviewed with application to a case study using a Canadian lens of approved treatment.

Conclusions: A solid understanding in a high-risk myelodysplasia is required for nurses to be able to help educate patients and caregivers on different strategies that are critical for success.