Myelodysplastic/Myeloproliferative Neoplasm - Clinical / Biology

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LONG-TERM INTERVENTION EFFECTS ON BONE MARROW MORPHOLOGY IN MYELOFIBROSIS: PATIENTS TREATED WITH RUXOLITINIB AND BEST AVAILABLE THERAPY

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Background: Myelofibrosis (MF) is a myeloproliferative neoplasm associated with splenomegaly, debilitating symptoms, progressive bone marrow (BM) fibrosis, and shortened survival. With the exception of BM transplantation, no therapy has shown a significant impact on disease progression. Ruxolitinib is an oral JAK1/JAK2 inhibitor that has demonstrated improvements in spleen volume, symptoms, and survival in patients (pts) with MF.
Aims: This study was conducted to gain insight into the effects of long-term ruxolitinib treatment on BM morphology in pts with MF and compare them to those seen in a best available therapy (BAT) cohort.
Methods: Trephine biopsies were obtained at baseline, 24 (68 pts) and 48 (18 pts) months (mo) from MF pts treated at MD Anderson Cancer Center who participated in a phase I/II trial of ruxolitinib (NCT00509899). The details of the study design and clinical outcomes have been published previously [Verstovsek, NEJM 2010]. Two of the authors (JT and H-MK) independently quantified BM fibrosis according to the World Health Organization (WHO) grading scale (0-3), and a third author (CB-R) reviewed the findings. Consensus decided discordant scores. Reviewers were blinded to pts characteristics and outcomes. WHO-defined BM fibrosis grading was also determined in prospectively collected specimens within a German, multicenter, observational database from a cohort of 139 pts (160 biopsies) treated with BAT for 24 (97 pts) and 48 (63 pts) mo. BAT included hydroxyurea [HU] (47%), interferon-alpha (7%), or assorted sequential therapies (25%). No active – or only supportive – therapy was given in 21% of the BAT cases. Biopsy intervals in the ruxolitinib-treated pts were defined per protocol; in the BAT cohort, biopsies were mostly performed based on a given patient's change in clinical condition at the discretion of the treating physician.
Changes in fibrosis grade vs. baseline were calculated for all time points and categorized as improvement, stabilization, and worsening. Additional analyses on changes over time in the degree of collagen deposition, amount of osteosclerosis, and BM cellularity were performed only in the ruxolitinib-treated cohort.
Results: At baseline, 21% of the ruxolitinib-treated cases presented with WHO-defined fibrosis grade 1, 53% with grade 2, and 26% with grade 3. At baseline, in this group, accumulation of collagen fibers was observed in 32 cases (47%): 30% with mildly increased (grade 1) and 17% with manifest or intense (grade 2 or grade 3) collagen deposition. About half of these pts had appreciable baseline osteosclerosis (grade 1: 32%; grade 2: 9%; grade 3: 9%). There were no significant differences in the distribution of baseline WHO-defined fibrosis grades between the ruxolitinib and BAT groups (P=0.441 by Cochran–Mantel–Haenszel test).
A higher percentage of ruxolitinib-treated pts showed stabilization or improvement in WHO-defined BM fibrosis at both the 24 and 48 mo time points vs. BAT pts. Worsening in fibrosis was significantly more prevalent in the BAT cohort at both time points.
Summary / Conclusion: This analysis of the effects of ruxolitinib in MF provides evidence that long-term therapy with this JAK inhibitor may meaningfully retard advancement of BM fibrosis. A comparable effect was not seen with long-term BAT. These results expand upon earlier observations using the same approach but with a smaller control cohort, consisting of pts treated with HU alone. Additional research is needed to elucidate the positive impact of JAK inhibition on BM morphology.

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THE INTERFERON SCORE TOWARDS PREDICTION OF RESPONSIVNESS TO INTERFERON ALPHA IN ESSENTIAL THROMBOCYTHEMIA

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Background: Interferon-alpha 2 (IFN) is able to induce hematological response in about 70-80% of ET patients but some of them could be defined as bad responders.
IFN binding its receptor results in tyrosine cross-phosphorylation and autophosphorylation of the JAKs proteins (Tyk2 and Jak1). These phosthyrosines recruit and activate STAT family member such as STAT1 and STAT3. These proteins induce the transcription of SOCSs, whose role is to extinguish cytokine signaling by inhibition of JAK kinase-activity directly through the KIR-domain, and indirectly promoting the proteasomal degradation of Jak2, by SOCS-box-motif. In summary, IFN induces the expression of SOCSs, which inhibit TPO mediated signaling through Jak2 double inhibition. This allows IFN-α and TPO pathway to cross-talks by means of the JAK-STAT-SOCS cascade.
Aims: In order to identify molecular markers that discriminate responders from non-responders to IFN, we analyzed bone marrow cells transcript levels of specific genes involved in the IFN receptor pathway, which signal cross-talks with the JAK-STAT pathway under TPO receptor. In particular we investigated the mRNA expression of JAK1, TYK2, STAT1, STAT3, SOCS1 and SOCS3.
Methods: We analyzed 60 ET patients treated with 3 million units of IFN-α-2b 5 times a week as induction (3 months), and 3 times a week as maintenance. Two groups of response were identified: *Good-Responders(R)* (n=44), who achieved complete response according to European Leukemia Net criteria, and *Bad-Responders(NR)* (n=17) who failed. The mRNA expression of genes of interest was measured in bone marrow samples from ET patients by RTq-PCR and tested for their predictive value using receiver operating characteristics (ROC) curves. Data were normalized as following: [mRNA normalized copy number (NCN)=mRNA target gene/mRNA GUSB*104]. An IFN score was calculated as an average in log2 of mRNA levels of genes differently expressed between *Good-R* and *Bad-R*.
Results: Main clinical characteristics were similar between the two groups of response. JAK2 V617F mutation was detected in 56,8% of *Good-R* and 58,8% of *Bad-R* (P=0,81) and no difference was found in JAK2V617F allele burden (P=0,17) and mRNA expression (P=0,2). Patients showed a median spleen volume of 500 mL in *Good-R* and 250 ml in *Bad-R* group (P=0.01). *Bad-R* compared with *Good-R* showed higher mRNA expression of JAK1 (134465 vs 44647; P<0.00001), STAT3 (49210 vs 23959; P=0.0002) and SOCS3 (18667 vs 10361; P=0,015). The AUC, using the normalized gene expression values, was 0.88 for JAK1, 0.81 for STAT3 and 0.7 for SOCS3. Average expression in log2 of these three genes was calculated and used as IFN score. The analysis reveled an AUC of 0.9 for this IFN signature (P<0,00001). The optimal cut-off point for IFN score to discriminate between *Good-R* and *Bad-R* was 15,75 and produced a sensitivity of 94,1%, specificity of 88,6% and likelihood ratio of 9.
Summary / Conclusion: We identified this set of three genes whose expression status could be translated into IFN score that showed a significant correlation with response outcome in ET.
Therefore, IFN score could represent a predictive biomarker for responsiveness to IFN and is likely to become a substantial aid to the physician, taking the paradigm of tailored therapy one step further, especially in chronic diseases such as ET.

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DEVELOPMENT AND CHARACTERIZATION OF A MURINE MODEL FOR LEUKEMIC TRANSFORMATION OF MYELOPROLIFERATIVE NEOPLASMS FOR PRECLINICAL THERAPEUTIC STUDIES

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Background: Transformation to acute myeloid leukemia (AML) represents a major clinical consequence of the Philadelphia-chromosome negative myeloproliferative neoplasms (MPNs) (Polycythemia Vera (PV), Essential Thrombocythemia (ET), and Primary Myelofibrosis (PMF)). Leukemic transformation (LT) after MPN occurs in as many as 23% of PMF patients within 10 years of diagnosis, and 4-8% of PV and ET patients develop AML in the first 18 years after diagnosis. The development of a post-MPN AML is associated with a poor clinical outcome, and characterized by a poor response to conventional anti-leukemic therapies. Although somatic mutations in the JAK-STAT signaling pathway occur in the majority of MPN patients, the somatic mutations that drive LT from a pre-existing MPN have not been fully delineated. Recent candidate mutational studies have identified recurrent somatic mutations in a subset of known leukemogenic disease alleles at the time of transformation from MPN to AML, including mutations in *TP53, IDH1/2, TET2* and *SRSF2.* However, the functional contribution of these specific genetic events to LT has not been delineated
Aims: To develop a genetically accurate murine model of LT, in order to further understanding of progression from MPN to AML and to use this preclinical model to test novel therapeutic approaches.
Methods: Expression of the *JAK2*V617F mutation in combination with *Tp53* loss was modeled *in vivo.* Bone marrow (BM) from C57/Bl6 *Tp53*$^{-/-}$ and littermate control mice was infected with *JAK2*V617F-IRES-GFP retrovirus, followed by transplantation of transduced cells into lethally irradiated congenic recipients. Outcomes measured included: survival, peripheral blood counts, organ weights, flow cytometry analysis of bone marrow and spleen derived cells, and morphologic evaluation of bone marrow and spleen.

Results: Transplantation of *JAK2*V617F/*Tp53*$^{-/-}$ cells, but not *JAK2*V617F positive cells was associated with impaired survival; 50% of mice injected with *JAK2*V617F/*Tp53*$^{-/-}$ cells died by day 100, whereas all mice injected with *JAK2*V617F positive cells survived 100 days or longer (P=0.011) (figure1). Mice injected with *JAK2*V617F/*Tp53*$^{-/-}$ cells presented with significant leukocytosis, with a mean WBC of 38.4 in mice engrafted with *JAK2*V617F/*Tp53*$^{-/-}$ cells compared with 11.4 in *JAK2*V617F/*Tp53* wildtype mice (no p value). At the time of sacrifice, all mice engrafted with *JAK2*V617F/*Tp53*$^{-/-}$ cells had increased numbers of blasts in the peripheral blood and bone marrow, as assessed by morphologic evaluation and flow cytometric analysis of CD117 expression. The disease from *JAK2*V617F/*Tp53*$^{-/-}$ cells, but not *JAK2*V617F positive cells, was transplantable into secondary recipients consistent with increased self-renewal *in vivo*. Flow cytometric analysis of spleen and bone marrow derived cells from leukemic mice demonstrated an increased percentage of megakaryocyte-erythroid progenitors (MEPs) and erythroblasts. *In vitro* assays and *in vivo* studies in secondary transplantation studies were carried out using this model. INCB18424 and CYT 387 exposure resulted in dose-dependent inhibition of colony formation *in vitro*. *In vivo* testing of INCB18424 and the HSP90 inhibitor PU-H71 was carried out in secondary recipients and the results of this preclinical trial will be presented.

Summary / Conclusion: The expression of *JAK2*V617F plus *Tp53* loss, a genotype commonly seen in patients who transform to AML after MPN, efficiently models LT *in vivo*. This model can now be utilized to assess the leukemic cell of origin in transformed disease, and to test novel therapeutic strategies which will inform clinical trials in this poor-risk hematologic malignancy.

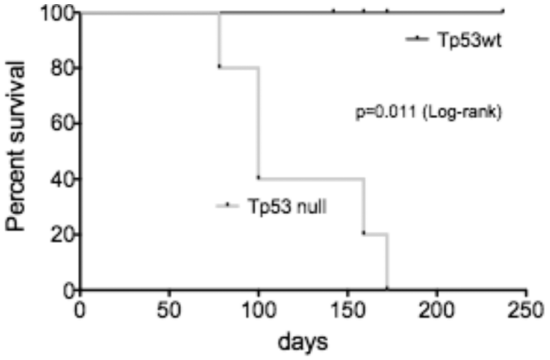

Figure 1.

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INCIDENCE, TREATMENT AND SURVIVAL OF MYELODYSPLASTIC SYNDROMES: A POPULATION-BASED STUDY OF 5,144 PATIENTS DIAGNOSED IN THE NETHERLANDS FROM 2001 TO 2010

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Background: At the beginning of the new millennium, myelodysplastic syndromes (MDS) were formally classified as malignant myeloid neoplasms by the World Health Organization (WHO), and consequently MDS became reportable malignancies to population-based cancer registries as of 2001. To date, studies on incidence and survival in MDS based on data from population-based cancer registries are scarce. Further, studies regarding treatment decisions in the entire MDS population are not available.

Aims: We conducted a nationwide population-based study to assess trends in incidence, initial treatment and survival among newly diagnosed patients with MDS during a 10-year period in the Netherlands.

Methods: Morphology codes of the International Classification of Diseases for Oncology Third Edition (ICD-O-3) were used to identify newly diagnosed patients with MDS from the nationwide Netherlands Cancer Registry (NCR) from 2001 to 2010 with follow-up to 2011. All MDS subtypes according to the ICD-O-3 were included in the NCR, namely refractory anemia (RA), RA with ringed sideroblasts (RARS), MDS with isolated del(5q) (5q- syndrome), refractory cytopenia with multilineage dysplasia (RCMD), RA with excess blasts (RAEB) and MDS, not otherwise specified. Age-standardized incidence rates (ASRs) of MDS were calculated per 100,000 person-years. Relative survival rates (RSRs) were calculated as a measure of disease-specific survival. If initial treatment was started within 9 months after diagnosis, it was recorded in the NCR.

Results: We identified a total of 5,144 newly diagnosed patients with MDS from January 1, 2001 to December 31, 2010 (median age 74 years). Of these patients, 486 (9%) were classified as RA, 583 (11%) RARS, 82 (2%) the 5q-syndrome, 524 (10%) RCMD, 966 (19%) RAEB, while 2,503 (49%) were unspecified. Interestingly, the proportion of unspecified MDS decreased from 60% in 2001 to 36% in 2010. The reported number of patients diagnosed with MDS increased throughout the study period; however, the annual ASR reached a plateau at 2.8 since 2007. Men had a higher overall incidence than women (3.3 *v* 1.7). The age-specific incidence of MDS during the entire study period increased in parallel with older age, from 0.3 among those aged 50 years or younger to 28.5 among those aged 80 years or older. Of all patients, 4,562 (89%) did not receive treatment or only received supportive care, 348 (7%) received chemotherapy and 74 (1%) received chemotherapy followed by a stem cell transplantation. Survival in MDS did not improve over time. All MDS subtypes had inferior survival compared with the expected survival in the general population (Figure 1A). Five-year RSRs were 53% in patients with RA, 58% in patients with RARS, 48% in patients with the 5q- syndrome, 38% in patients with RCMD, 18% in patients with RAEB, and 39% in patients with an unspecified MDS. Age at diagnosis was an important predictor for survival as RSRs decreased in parallel with older age (Figure 1B). Five-year RSRs were 59% for patients aged 18-49 years, 52% for patients aged 50-59 years, 41% for patients aged 60-69 years, 36% for patients aged 70-79 years, and 29% for patients aged ≥80 years. The RSRs between both sexes were comparable.

Summary / Conclusion: The incidence of MDS in the Netherlands is similar to other Western European countries and increased over time due to improved notification. Morphological assessment of MDS according to the WHO classification seems challenging as almost half of the recorded MDS cases were unspecified. Nevertheless, the proportion of specified cases increased over time, which was presumably due to better knowledge about the WHO classification of MDS. The lack of improvement in patient survival might be explained by the limited availability and use of therapeutic agents. Therefore, more emphasis is needed to improve current treatment strategies and to develop new treatment options in MDS.

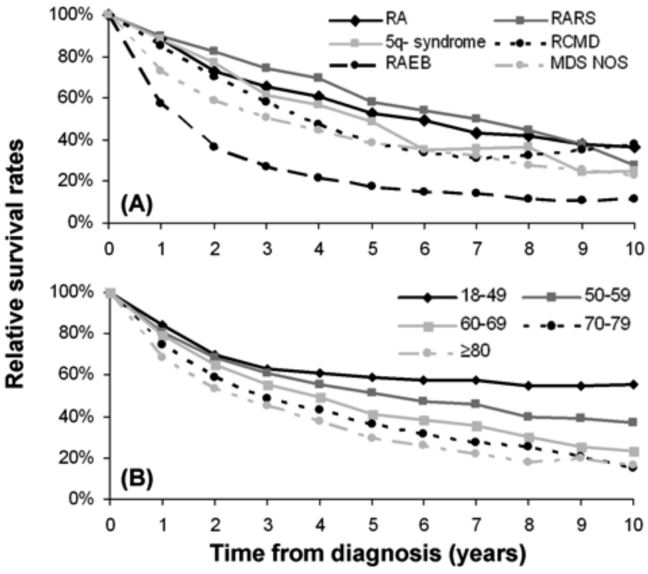

Figure 1. Cumulative relative survival among patients diagnosed with myelodysplastic syndromes in the Netherlands by (A) subtype and (B) age at diagnosis throughout the study period from 2001 to 2010.

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A PILOT PHASE ONE DOSE FINDING SAFETY STUDY OF A THROMBOPOIETIN-RECEPTOR AGONIST, ELTROMBOPAG, IN PATIENTS WITH MYELODYSPLASTIC SYNDROME TREATED WITH AZACITIDINE

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Background: Thrombocytopenia is a common manifestation of Myelodysplastic Syndroms (MDS) and is an independent adverse prognostic factor for survival. Azacitidine (Aza) is today the treatment of choice for patients with high-risk MDS not eligible for stem cell transplantation. In the pivotal randomized studies with Aza, grade 3-4 thrombocytopenia was reported in up to 85% of patients, and the main causes of Aza discontinuation were hematological adverse events. In parallel to the use of growth factors such as Erythropoietin and G-CSF in MDS, stimulating platelet production by a thrombomimetic agent may be an attractive alternative to platelet transfusions. Eltrombopag is a novel thrombomimetic agent that activates the thrombopoietin receptor (TPO-R) and stimulates megakaryopoiesis and thrombopoiesis. Studies with eltrombopag in leukemia cell lines did not show enhancement of leukemia cell proliferation, instead inhibition of leukemia cells was observed in the majority of tested cell lines.
Aims: In this Phase I Pilot Study (ClinicalTrials.gov identifier: NCT01481220) we explored the safety and tolerability of combining eltrombopag with Aza in the treatment of patients with high-risk MDS.
Methods: Patents with high-risk MDS eligible to treatment with Aza according to the labeled indication and thrombocytopenia (Platelet counts $<75x10^9$/L) were included. Cohorts of three patients received Aza in combination with increasing doses of eltrombopag tablets (50-100 mg, 100-200 mg, 200-300mg and a final cohort with 300mg as an unchanged dose) during 3 Aza cycles (13 weeks). Patient monitoring included repeated blood and bone marrow samples.
Results: Eleven patients, with a median age of 71 (range 53-83) have been included. Reported severe adverse events include one bacterial bronchitis (cohort 1, eltrombopag 50-100 mg), recurrent erysipelas (one patient in cohort 3, eltrombopag 200-300 mg), one case of fatal pneumonia with E. coli septicemia (Cohort 3) and one deep vein thrombosis, elevated liver enzymes and progressive disease occurring in a patient with highly proliferative MDS-AML (Cohort 3). This dose-cohort is now being completed with 3 more patients. Platelet counts improved or remained stabile in 9/11 patients despite Aza treatment while two patients remained dependent on platelet transfusion. Median platelet counts were 40, 51 and 64 x 10^9/L at inclusion, after one and after three Aza cycles, respectively. Bleeding symptoms were uncommon. No MDS disease progressions related to study medication were reported. Three more patients are to be recruited to the final cohort and updated results will be presented during the 18th EHA congress.
Summary / Conclusion: The combination of eltrombopag with Aza in high-risk MDS patients with thrombocytopenia is feasible and well tolerated. Improvements in platelet counts and anti-leukemic effect through adding eltrombopag cannot be excluded and needs to be explored in a phase-two study.