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Background: Chronic myelomonocytic leukemia (CMML) is characterized by increased proliferation and myelomonocytic lineage commitment of hematopoietic stem cells (HSCs). Mutations in the RAS-pathway occur frequently in CMML patients and lead to a CMML-like myeloproliferative disorder (CMML-MPD) in mice via causing hypersensitivity to GM-CSF. Loss of RAF kinase inhibitor protein (RKIP), a negative regulator of RAS signaling, is frequent in myelomonocytic and monocytic subtypes of acute myeloid leukemia (AML) and is often associated with RAS mutations. Moreover, RKIP loss has recently been shown to increase the proliferation of AML cell lines.

Aims: In this work, we aimed at investigating the role of RKIP in the development of CMML.

Methods: RKIP expression was measured by immunoblot and quantitative real-time PCR in 23 primary CMML patient samples as well as in CD34+ HSCs, B-lymphocytes, granulocytes and monocytes of four healthy donors. Sequence analysis of CMML samples was done with an Ion Torrent Next Generation Sequencing platform using an amplicon panel covering 39 genes recurrently mutated in myeloid neoplasms. Effects of RKIP on GM-CSF-induced myelomonocytic differentiation were studied in human CD34+ HSCs lentivirally transduced with RKIP shRNA, as well as in a genetic mouse model for RKIP deletion (RKIP^{-/-}). Effects of RKIP on CMML development were initially studied in the same RKIP^{-/-} model. Additionally, these mice were crossed with animals exhibiting a somatically inducible mutation in NRAS (RKIP^{-/-}Mx1-Cre-NRAS^{G12D}) and the severity of CMML-MPD onset was studied at an age of six months.

Results: Loss of RKIP protein expression was observed in 6/23 (26%) CMML patient specimens and was associated with decreased mRNA levels as well (P<0.001). Patients with RKIP loss exhibited an increased percentage of myelomonocytic cells in the peripheral blood (86% vs 75%; P=0.0226). One or more mutations affecting the RAS signaling pathway were detected in all specimens with RKIP loss. In addition to the previously demonstrated induction of proliferation, we then aimed to delineate a role of RKIP loss in myeloid lineage commitment. When studying healthy donors, we observed that RKIP expression was high in HSCs and lymphoid cells, but significantly decreased in cells belonging to the myeloid lineage (monocytes, P=0.001 and granulocytes, P<0.001). In functional experiments, knockdown of RKIP increased the GM-CSF-induced myelomonocytic lineage commitment of both, human and murine HSCs (P<0.05 and P=0.0295, respectively). These results could be corroborated in-vivo, as intraperitoneal injection of GM-CSF caused a significant increase of myelomonocytic cells in the intraperitoneal cavity (P=0.006), bone marrow (P=0.007) and peripheral blood (P=0.027) in RKIP^{-/-} mice when compared to their wildtype littermates. In a final step, we evaluated the potential of RKIP loss to cause CMML-MPD in mice. While it proved to be insufficient to cause the disease as a single event in RKIP^{-/-} mice, it aggravated the CMML-MPD phenotype in animals carrying an additional mutation in NRAS. In this case, RKIP deletion caused worsening of leucocytosis (P=0.036) and splenomegaly (P=0.035), which was associated with increased levels of myelomonocytic cells in the bone marrow (P=0.028), peripheral blood (P=0.002) and spleen (P=0.025).

Summary/Conclusions: RKIP loss is a frequent event in CMML and is associated with mutations affecting the RAS signaling cascade. Loss of RKIP is functionally involved in myelomonocytic lineage commitment of HSCs and aggravates CMML-MPD development in mice carrying an additional mutation in NRAS.

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JAK2 V617F HAEMATOPOIETIC CLONES WITH DIFFERENT EXPANSION KINETICS ARE DETECTABLE SEVERAL YEARS PRIOR TO MPN DIAGNOSIS

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Background: JAK2 V617F is the most common somatic mutation in the classical myeloproliferative neoplasms (MPNs) and is also frequent amongst healthy individuals with age-related clonal haemopoiesis (ARCH).

Aims: To investigate the pre-clinical clonal evolution of MPNs.

Methods: We identified 12 individuals with JAK2 V617F mutant MPN from whom blood DNA was available from the time of MPN diagnosis and also from an earlier time point of between 4.5-15.2 years previously (median 10.2 years) when blood was donated for registration to the Cyprus Bone Marrow Donor Registry. We used deep DNA sequencing to interrogate all 24 samples at 15 myeloid mutation hotspots including JAK2 V617, using an established multiplex PCR/MiSeq sequencing protocol that reliably detects nucleotide substitutions present at a variant allele fraction (VAF) ≥0.008. Additionally, for 12 samples with sufficient DNA available, we performed targeted DNA capture for all exons of 41 genes recurrently mutated in myeloid neoplasms using a custom RNA-bait library followed by sequencing on Illumina HiSeq 2500. Finally, we genotyped archival Registry samples for the rs12343867 single nucleotide polymorphism (SNP) (C/T) linked to the JAK2 46/1 haplotype.

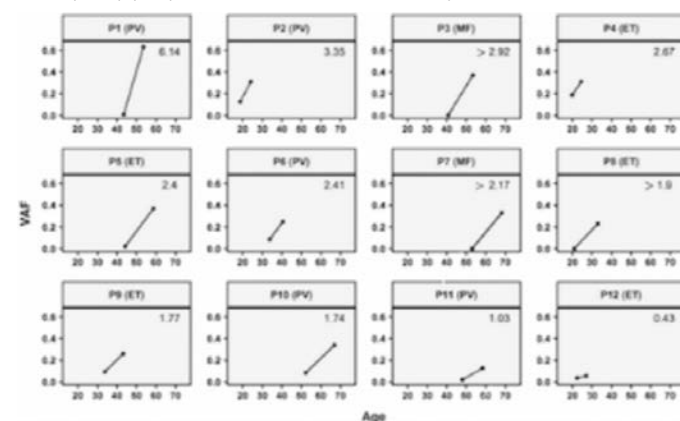


Figure 1. Pre-clinical expansion of JAK2 V617F clones in 12 MPN patients. VAF sizes of JAK2 V617F positive clones at the two time points against the age of participants at the time. The specific diagnosis is indicated in brackets next to each patient's ID and the average annual rise in VAF is indicated in the lower right quadrant of each plot. Samples P3, P7 and P8 had no detectable JAK2 V617F at time point A. PV = polycythemia vera, ET = essential thrombocythemia, MF = idiopathic myelofibrosis

Figure 1.

Results: Amplicon sequencing returned a median coverage of 6641 reads per nucleotide (nt) at the studied hotspots. This confirmed the presence of JAK2 V617F in all 12 diagnostic and 9 of 12 archival samples. The remaining 3 samples were JAK2 V617F negative at the sensitivity of our assay (VAF≥0.008). The only other hotspot mutation identified was SRSF2 P95R in one patient, P3, whom had a diagnosis of myelofibrosis. Pulldown sequencing of all exons of 41 genes from 12 samples with sufficient DNA returned an average coverage of 1978 reads per nt and showed a close correlation in JAK2 V617F and SRSF2 P95R VAF quantifications with amplicon sequencing. The JAK2 V617F VAF at MPN diagnosis differed between patients as expected, however the average rate of clonal growth also varied widely between individuals, ranging from 0.36 to 6.2% per annum (Figure 1). Targeted exon capture from 12 of 24 samples, only identified one co-mutation with a VAF >0.02, the SRSF2 P95R in patient P3. As this locus was also amplified by amplicon sequencing, we were able to quantify the SRSF2 P95R VAF in both the diagnostic and the archival DNA sample taken 12.6 years earlier. In the P3 diagnostic sample the VAFs for JAK2 V617F and SRSF2 P95R were similar (0.37 and 0.41 respectively) indicating that they co-occurred in most cells of the neoplastic clone. In the archival sample from P3, the SRSF2 P95R was detectable at a VAF of 0.06, however the JAK2 V617F was absent/undetectable at the sensitivity of our method (VAF≥0.008) indicating the SRSF2 P95R was the clone-founding mutation in this neoplasm. The genotyping results for the rs12343867 SNP revealed a tentative association in our small cohort between homozygosity for the risk allele (C) linked to the JAK2 46/1 haplotype and the average annual increase in JAK2 V617F VAF. This will need to be verified in larger studies.

Summary/Conclusions: Our findings reveal that JAK2 V617F neoplasms develop from clonal haematopoiesis over many years. The rate of clonal expansion of JAK2 V617F clones in the pre-clinical phase was highly variable and although it was tentatively associated with the 46/1 haplotype, the high variability observed suggests that other factors likely influence clonal progression.

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DISRUPTION OF HAEMATOPOIETIC STEM CELL HETEROGENEITY IN A MOUSE MODEL OF MYELOPROLIFERATIVE NEOPLASM

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Background: The hematopoietic stem cell (HSC) compartment in mice encompasses a broad range of heterogeneous cell types including highly lineage-biased HSCs, such as platelet-biased HSCs (PMID:23934107). Myeloproliferative neoplasms (MPNs) are a heterogeneous spectrum of clonal hematopoietic disorders, that includes essential thrombocythemia (ET), a MPN-subtype usually presenting with isolated thrombocytosis. Most ET patients carry a gain-of-function point mutation in JAK2 (JAK2V617F), with several other collaborating hits reported to co-occur with JAK2V617F at lower frequencies, including loss-of-function mutations of the epigenetic regulator EZH2, which are more frequent in advanced MPN.

Aims: Although it is broadly accepted that MPNs are propagated by counterparts of HSCs, the impact of collaborating MPN-associated mutations arising in different HSC subsets remains unclear. We aimed to explore the possibility that platelet-biased HSCs might selectively promote development of an ET phenotype.

Methods: We generated a novel mouse model of MPN that carries a conditional knock-in of heterozygous human JAK2V617F (hJAK2V617F) and the conditional knock-out (KO) of EZH2 together with an inducible Mx1-Cre transgene. To analyse platelet-biased HSC subsets upon onset of the mutation(s), we also crossed in the vwf-eGFP transgene, which is selectively expressed in platelet-biased HSCs.

Results: Compared to wild-type and single mutant mice, EZH2-KO hJAK2V617F mice showed increased platelet counts, including a subset of mice which became acutely unwell with an extreme thrombocytosis. Strikingly, in serial bone marrow (BM) transplantation assays, EZH2-KO fully rescued the previously described hJAK2V617F-associated transplantation defect (PMID:20489053). EZH2-KO hJAK2V617F BM recipients showed long-term serial engraftment that was fully restricted to the platelet and myeloid lineages with a persistent thrombocytosis and absence of lymphoid reconstitution. RNA-sequencing revealed upregulation of several signaling pathways, including Hedgehog, and increased inflammation associated gene expression in EZH2-KO hJAK2V617F HSCs. Unexpectedly in this mouse model of thrombocytosis, phenotypic analysis of the HSC compartment in the BM showed that vwf-eGFP+ve HSCs were selectively lost (fold change[FC]=0.12 p=0.009), while vwf-eGFP-ve HSC numbers remained unaffected (FC=1.06 p=0.88) in Ezh2-KO hJAK2V617F mice. To assess a differential contribution of vwf-eGFP+ve HSCs vs vwf-eGFP-ve HSCs in the ability to propagate MPN, we sorted HSCs according to vwf-eGFP expression and transplanted them into recipient mice. Unlike their normal counterparts, which showed lymphoid-biased reconstitution, vwf-eGFP-ve HSCs from Ezh2-KO hJAK2V617F mice primarily gave rise to platelets and myeloid cells. In contrast, vwf-eGFP+ve HSCs from Ezh2-KO hJAK2V617F mice engrafted poorly without recapitulating the disease in recipients.

Summary/Conclusions: In this novel Ezh2-KO hJAK2V617F mouse model, Ezh2 loss collaborates to worsen thrombocytosis and rescue the HSC function defect in hJAK2V617F mice. We also observed a striking disruption of phenotypic and functional HSC heterogeneity in Ezh2-KO hJAK2V617F mice with an unexpected and selective loss of vwf-eGFP+ve HSCs together with subversion of vwf-eGFP-ve HSCs towards platelet-myeloid lineage commitment. This previously undescribed disruption of HSC heterogeneity in myeloid malignancy together with the clonal advantage conferred to HSCs by EZH2-KO helps to explain how this collaborating mutation might promote the development of more advanced MPN.

Clinical trials including treatment discontinuation in CML

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DASATINIB IN CHILDREN AND ADOLESCENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP) FROM A PHASE 2 TRIAL

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Background: As safe and effective frontline treatment options for children and adolescents with CML are limited, and no approved therapies exist for patients (pts) resistant/intolerant to imatinib (IM), additional treatment options and alternative formulations are greatly needed for this younger population. Dasatinib (DAS) has proven efficacy in adults with newly diagnosed CML-CP, as well as those resistant/intolerant to IM (Cortes JCO 2016, Shah AJH 2016). Results of a phase 1 study confirmed its dosing and safety in pediatric pts (Zwaan JCO 2013); however, a larger prospective study is necessary to further support the use of DAS in pediatric pts with newly diagnosed or IM-resistant/intolerant CML-CP.

Aims: To determine whether DAS is safe and effective in pediatric pts with CML-CP newly diagnosed or resistant/intolerant to IM enrolled in a phase 2, open-label, nonrandomized prospective clinical trial (CA180-226/NCT0077 7036).

Table 1.

	Treated pts with CML-CP (n=113)		
	IM-resistant/intolerant CML-CP (n=29)	Newly diagnosed CML-CP (n=84)	
		Tablet (n=51)	PFOS (n=33)
Median average daily dose, mg/m ² (range)	58 (35-72)	59 (37-78)	65 (38-99)
Median duration of treatment, months (range)	50 (2-90+)	52 (8-75+)	27 (<1-42+)
Pts on treatment, n (%)	14 (48)	61 (73)	
		37 (73)	24 (73)
Reasons for discontinuation, n (%)			
Progressive disease	5 (17)	5 (10)	1 (3)
Study drug toxicity	0	0	1 (3)
Pt withdrawal*	3 (10)	2 (4)	1 (3)
Maximum clinical benefit	2 (7)	1 (2)	0
Noncompliance	1 (3)	0	0
Other	4 (14)	6 (12)	6 (18)
MCyR, ^a % (95% CI)		57 (46, 68)	
By 3 months	55 (36, 74)	55 (40, 69)	61 (42, 77)
By 12 and 24 months	90 (73, 98)	96 (90, 99)	
		98 (90, 100)	94 (80, 99)
Median time to MCyR, ^a months (95% CI)	3.1 (2.8, 4.1)	3.0 (2.9, 4.3)	
		3.3 (2.9, 5.6)	3.0 (2.8, 5.0)
CCyR, ^b % (95% CI)		64 (53, 74)	
By 6 months	66 (46, 82)	61 (46, 74)	70 (51, 84)
By 12 months	76 (57, 90)	92 (84, 97)	
		94 (84, 99)	88 (72, 97)
By 24 months	83 (64, 94)	94 (87, 98)	
		96 (87, 100)	91 (76, 98)
Median time to CCyR, ^b months (95% CI)	3.9 (2.8, 5.6)	5.6 (5.0, 6.0)	
		5.7 (3.7, 6.2)	5.6 (3.1, 6.0)
MMR, % (95% CI)		52 (41, 63)	
By 12 months	41 (24, 61)	57 (42, 71)	46 (28, 64)
By 24 months	55 (36, 74)	75 (60, 86)	
		75 (60, 86)	64 (45, 80)
Estimated 48-month PFS, % (95% CI)	78 (57, 89)	93 (83, 97)	
		92 (80, 97)	97 (79, 100)

*Refers to either study treatment discontinuation by pt, or withdrawing consent from further follow-up.
^aResponses based on ≥20 metaphases.
CI=confidence interval; MMR=major molecular response.