Ruxolitinib and interferon-α2 combination therapy for patients with polycythemia vera or myelofibrosis: a phase II study

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Supplemental methods, figures, and tables

Expanded methods

Study design

The COMBI study (#EudraCT2013-003295-12) was an investigator-initiated, multicenter, open-label, single-arm phase II study; we included 50 patients: 32 with PV and 18 with MF. The study was conducted 2014–2018 at three sites in Denmark and approved by the Danish Regional Science Ethics Committee and the Danish Medicines Agency. It was done under the principles of the Declaration of Helsinki. Patients gave written informed consent.

Inclusion/exclusion criteria

Patients aged ≥18 years with a PV or MF diagnosis according to the 2008 WHO criteria were considered eligible, if they had evidence of active disease, defined as one or more of the following: need for phlebotomy, white blood cell count ≥10 10^9/L, platelet count ≥400 10^9/L, constitutional symptoms, pruritus, symptomatic splenomegaly, and previous thrombosis. Patients fulfilling one or more of the following criteria were excluded: pregnancy; allergic hypersensitivity to study medications; ECOG performance status ≥3; other active malignancy within five years; impaired renal or hepatic function; treatment with immunosuppressive drugs except corticosteroids within the preceding six months; psychiatric disease; severe neurological disease; uncontrolled metabolic disease; severe cardiac disease; white blood cell count <1.5 10^9/L; and platelet count <100 10^9/L.

Treatment schedule and dosing

Initiation of study treatment and baseline evaluations were preceded by a minimum seven-day wash-out period after discontinuation of any previous cytoreductive therapies. Patients were initially treated with PEG-IFNα2a (Pegasys®; Genentech (Roche), South San Francisco, CA, USA) 45 µg/week or PEG-IFNα2b (PegIntron®; Merck Sharp & Dohme, Hertfordshire, UK) 35 µg/week subcutaneously and ruxolitinib (Jakavi®; Novartis, Basel, Switzerland) 5–20 mg BID orally depending on platelet count, according to the manufacturers dosing instructions. The patients were reintroduced to the last used type of PEG-IFNα2. The

medication was self-administered. Doses were modified at the investigators' discretion based on toxicity and efficacy.

Patient evaluations

Study visits were scheduled at baseline, two weeks, one month, three months, and every third month after that until two years of treatment. Each visit included documentation of adverse events, full-scale hematology, blood biochemistry investigations, Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF) (1), and assessment of compliance by research staff. Bone marrow biopsies were done at baseline and after one and two years of treatment. Spleen size, measured as longest diameter by sonography, and *JAK2* V617F or *calreticulin* (*CALR) mutation measurements* were done at baseline and after three months, six months, one year and two years. The proportion of *JAK2* V617F and *CALR* mutated alleles were quantified using a high-sensitivity real-time qPCR on whole-blood with a lower detection limit of ≤0.1% (2, 3).

Endpoints

The primary outcome was efficacy, based on hematological parameters, quality of life measurements, and the *JAK2* V617F burden. The 2013 ELN and IWG-MRT response criteria were used to assess efficacy (4, 5).

In brief, for patients with PV, achieving complete remission (CR) required resolution of disease-related symptoms and hepatosplenomegaly; peripheral blood count remission (PBCR), with a platelet count below 400 x 10^9/L and white blood cell count < 10 x 10^9/L and absence of leucoerythroblastosis; no progression of the disease and no hemorrhagic or thrombotic events; and bone marrow histological remission (BMHR), with age-adjusted normocellularity, and fibrosis grade ≤1. A partial remission (PR) required all the above except BMHR. A criterion for resolution of disease-related symptoms in patients with PV is 'large symptom improvement' defined as a ≥10-point decrease in MPN-SAF total symptom score (TSS). We modified this criterion for patients with a baseline TSS <10 (n=3). In these patients, durable TSS <10, and no disease-related symptoms reported at the study visit fulfilled the criteria.

For patients with MF, achieving CR required resolution of disease-related symptoms and hepatosplenomegaly; PBCR with hemoglobin ≥10 g/dL, neutrophil count ≥ 1 x 10^9, platelet count ≥100 x 10^9/L all below the

upper normal level, and <2% immature myeloid cells; and BMHR with age-adjusted normocellularity, fibrosis grade ≤1, and <5% blasts. A PR required resolution of disease-related symptoms and hepatosplenomegaly and either PBCR or BMHR. Clinical improvement (CI) was defined as an anemia response (≥20 g/L increase in hemoglobin level in transfusion-independent patients or transfusion-dependent patients becoming transfusion-independent) or symptoms response (≥50% reduction in MPN-SAF TSS). Spleen response was not included since we could not verify with MRI or CT scans. Progressive disease was defined as significantly increased splenomegaly or leukemic transformation (4, 5). Patients without a response or progressive disease was defined as having stable disease.

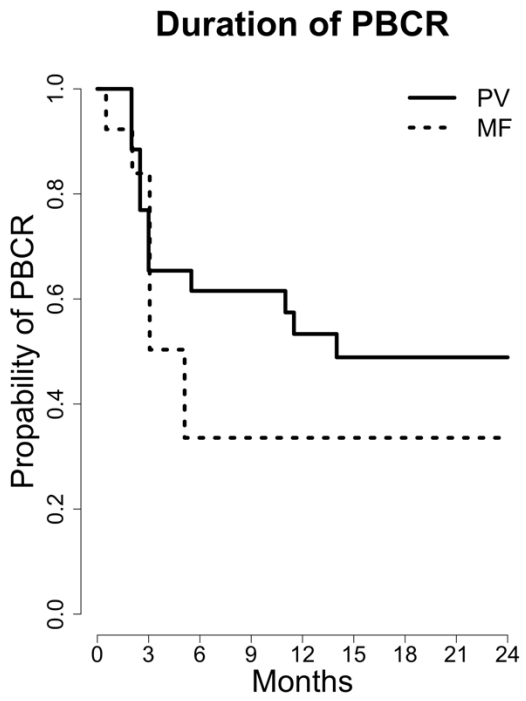
Changes in quality of life measurements were assessed using the MPN-SAF questionnaire. Molecular response (MR) was defined as either complete molecular response (CMR), defined as eradication of a preexisting abnormality, or partial molecular response (PMR), defined as a ≥50% decrease in *JAK2* V617F allele burden from baseline in patients with a baseline allele burden ≥20%. To compare with previous studies, we did an analysis with CMR set at ≤1% mutated alleles. Secondary endpoints included the change in spleen size and toxicity based on National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

Statistical analyses

We did the statistical analyses using R.3.2.3 (R Foundation for Statistical Computing, Vienna, Austria) and RStudio 1.0.136 (RStudio, Boston, MA, USA). Remission rates are presented with descriptive statistics. All patients initiating the study treatment were evaluated for response, similar to the modified intention-to-treat principle used for randomized controlled trials. Kaplan-Meier plots and log-rank tests were used when assessing time-to-event data. For two-by-two table analyses, chi-squared tests were used. For analyses of numerical repeated measurements, we used unstructured covariance or unstructured correlation generalized linear mixed models. Non-normally distributed variables were transformed using either logit transformation or log transformation and transformed back accordingly. For analyses of individual symptom scores,

we used generalized linear mixed models for ordinal outcomes. P-values <0.05 were considered statistically significant.

Supplemental figures and tables



Supplemental figure S1: Duration of first peripheral blood count remission (PBCR). Median duration of PBCR was 11 months. Of 21 patients that lost PBCR, 19 achieved PBCR again during the study period.

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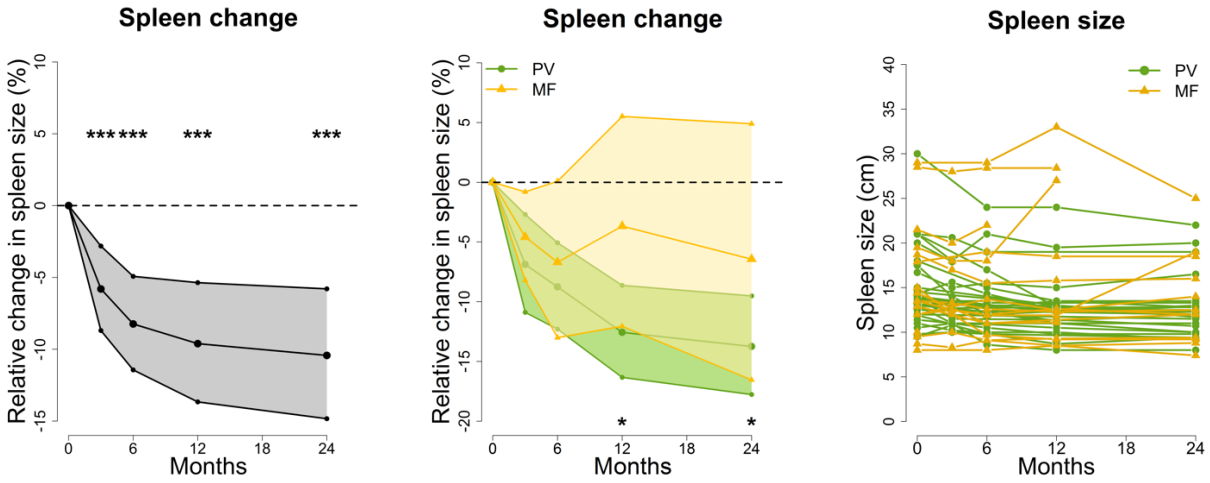
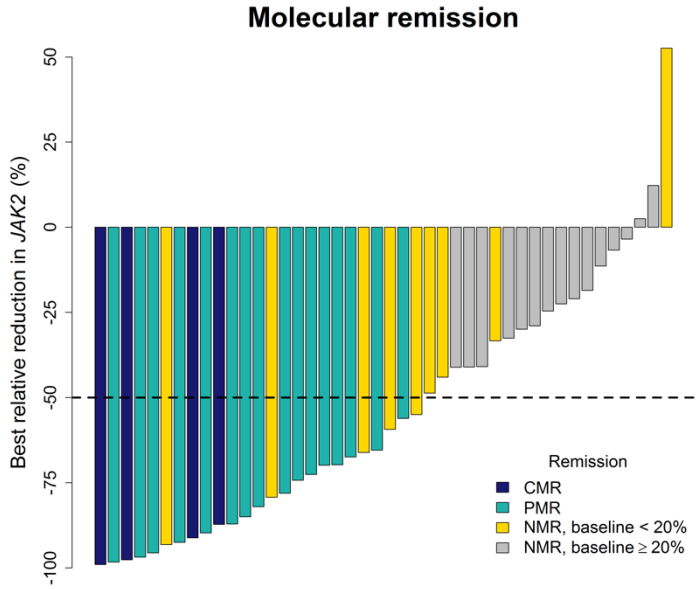


Supplemental figure S1: Change in spleen size. Relative change with 95% confidence interval from baseline in spleen size assessed by sonography (left), when stratified by diagnosis (middle), and individual changes (right).
* p-value <0.05
*** p-value <0.001



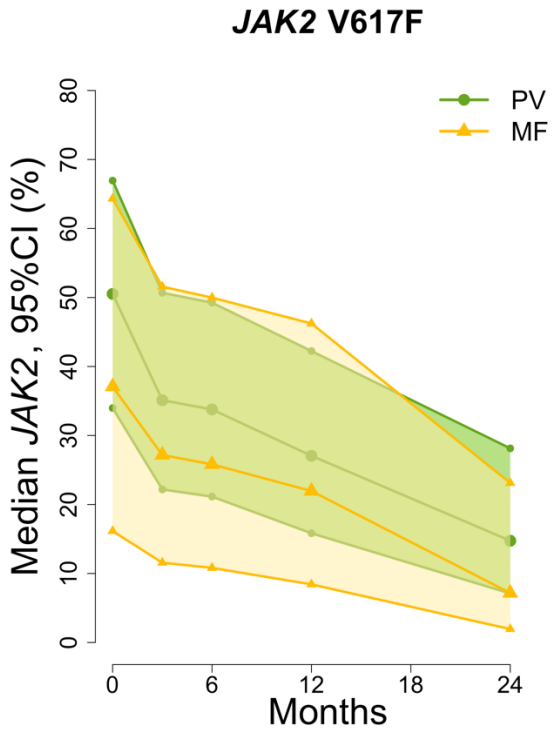
Supplemental figure S2: Best relative redution in *JAK2*-V61F allele burden. Waterfall plot over best relative reduction in *JAK2*-V617F allele burden (*JAK2*) with indication of molecular remission with complete molecular remission (CMR) defined as <1% mutated alleles. Partial molecular remission (PMR) is an ≥50% decrease in *JAK2* from baseline in patients with a baseline *JAK2* ≥20%.

NMR, no molecular remission

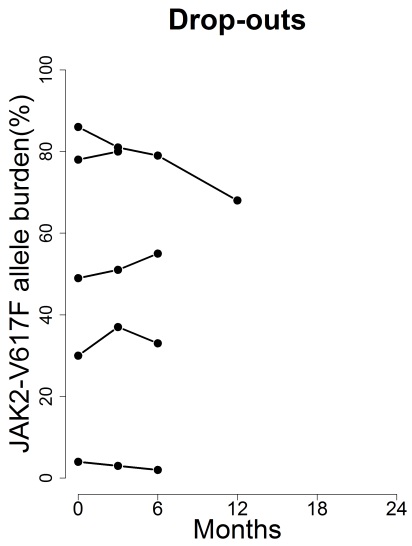
	CUT-OFF LEVEL FOR CMR					
MOLECULAR REMISSIOM	**Undetectable**	**<1%**	**<2%**	**<3%**	**<4%**	**<5%**
CMR	1 (2)	4 (8)	7 (14)	9 (18)	9 (18)	12 (24)
PMR	17 (34)	16 (32)	14 (28)	14 (28)	14 (28)	11 (22)
TOTAL MR	18 (36)	20 (40)	21 (42)	23 (46)	23 (46)	23 (46)

Supplemental table S1: Evaluation of molecular remission when setting the cut-off value of complete molecular remission to 1–5%.

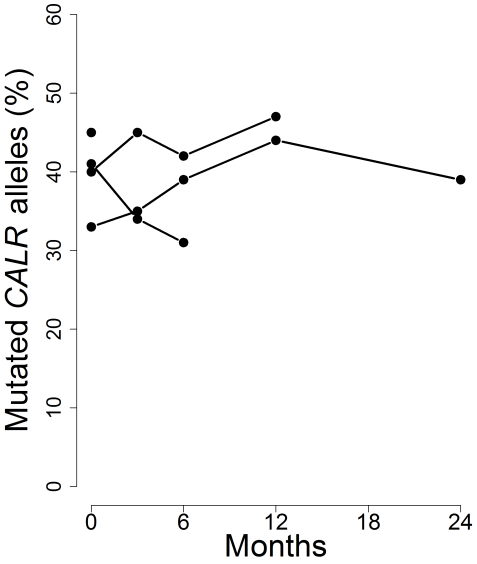
MR, molecular remission; CMR, complete molecular remission; PMR, partial molecular remission



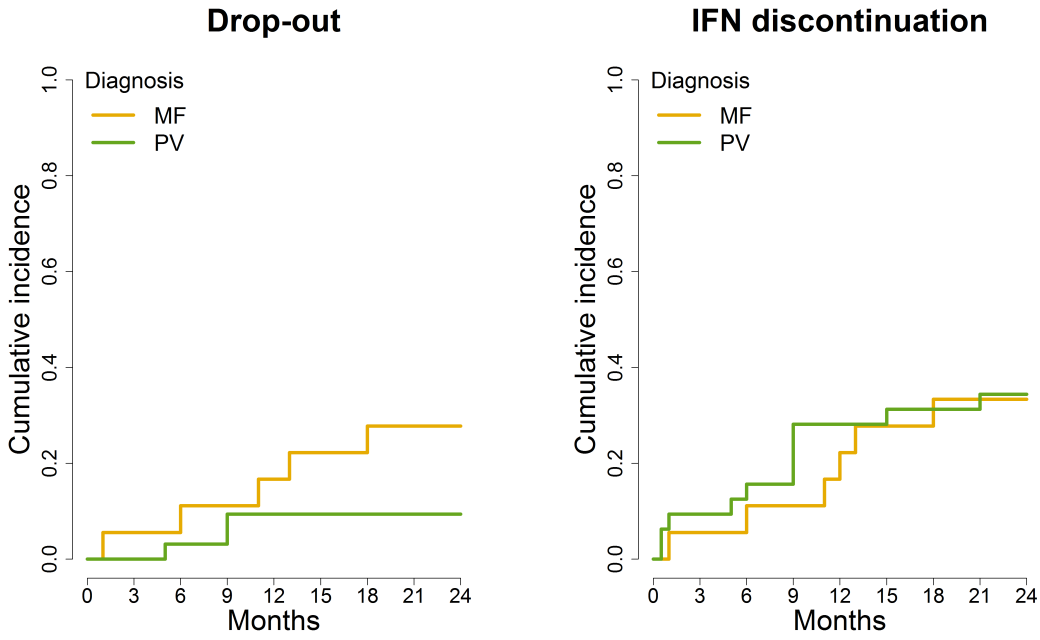
Supplemental figure S3: Change in *JAK2* V617F allele burden stratified by diagnosis. Change in median *JAK2*-V617F allele burden (*JAK2*) with 95% confidence interval in patients with PV patients and patients with MF.



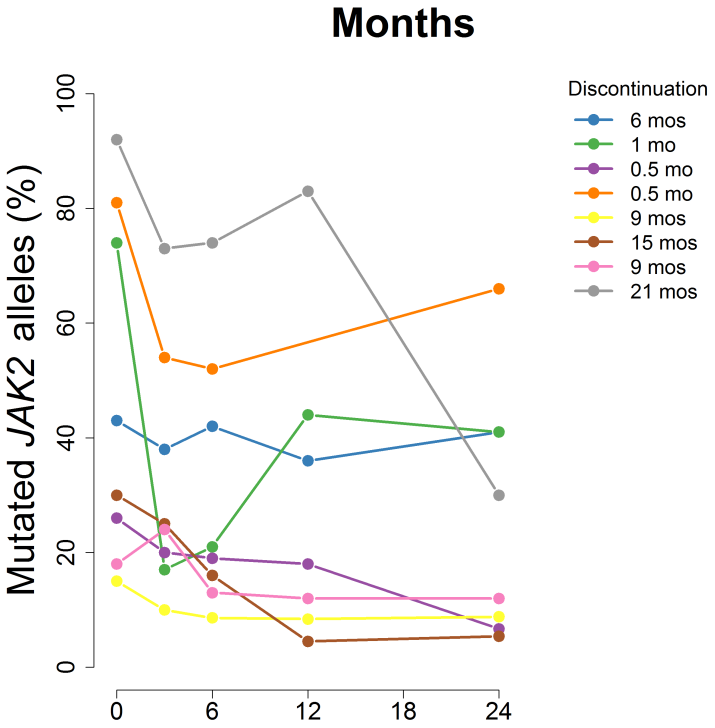
Supplemental figure S4: Change in *JAK2*-V617F allele burden in patients dropping out of the study.



Supplemental figure S5: Individual change in *CALR* allele burden



Supplemental figure S6: Drop-out rate (left) and rate of PEG-IFNα2 discontinuation (right).
PEG-IFNα2, pegylated interferon-α2; MF, myelofibrosis; PV, polycythemia vera



Supplemental figure S7: *JAK2* V617F allele burden measurements in participants who discontinued PEG-IFNα2.

Supplemental table S2: Reasons for discontinuation of study treatments

PATIENT NUMBER	DIAGNOSIS	DRUG(S) DISCONTINUED	REASON FOR DISCONTINUATION	TREATMENT TIME (MO.)	PRIOR TREATMENTS	INTOLERANT OR REFRACTORY TO STANDARD IFN TREATMENT
101	PV	PEG-IFNα2	Arthralgias	6	PEG-IFNα2, HU, ANA	Intolerant
102	PV	PEG-IFNα2	Angina Pectoris	1	PEG-IFNα2, HU, ANA	Intolerant
104	MF	Both	Neuropsychiatric symptoms	6	PEG-IFNα2, ANA	Intolerant
108	PV	Both	Infections and bleeding*	9	PEG-IFNα2, Imatinib	Intolerant
122	PV	PEG-IFNα2	Angina pectoris	0.5	PEG-IFNα2	Intolerant
124	PV	Both	Recurrent fever	5	PEG-IFNα2, HU	Refractory
127	MF	Both	Leukocytosis and thrombocytosis	18	PEG-IFNα2	Refractory
129	MF	Both	Increasing spleen size	11	PEG-IFNα2, HU	Intolerant
134	MF	Both	Anemia	13	PEG-IFNα2, HU	Refractory
135	PV	PEG-IFNα2	Thrombocytopenia	0.5	PEG-IFNα2, HU	Refractory
136	MF	PEG-IFNα2	Fatigue	12	PEG-IFNα2	Intolerant
137	PV	PEG-IFNα2	angioedema	9	PEG-IFNα2, HU	Intolerant
139	PV	Ruxolitinib	Leucopenia	12	PEG-IFNα2	Intolerant
142	MF	Both	Headache, dizziness, nausea, arthralgias, mood changes, angina pectoris	1	PEG-IFNα2, ANA, Busulphan	Intolerant
144	PV	PEG-IFNα2	Headache	15	PEG-IFNα2	Refractory
146	PV	PEG-IFNα2	Arthralgia and headache	9	PEG-IFNα2, HU	Intolerant
201	PV	PEG-IFNα2	Leucopenia	21	PEG-IFNα2, HU	Intolerant
302	PV	Both	Neuropsychiatric symptoms	9	PEG-IFNα2, HU	Both

*Multiple infections and gastrointestinal bleeding, requested to be taken off protocol
PV, Polycythemia vera; MF, Myelofibrosis; PEG-IFNα2, pegylated interferon-2α; HU, hydroxyurea; Anagrelide

Supplemental table S3: Main results stratified by the reason for discontinuation of PEG-IFNα2 before initiation of the trial.

	INTOLERANT (N=31)	REFRACTORY (N=10)	BOTH (N=6)	NAÏVE (N=3)
POLYCYTHEMIA VERA, N (%)	19 (61)	7 (70)	4 (67)	2 (67)
DROP-OUT, N (%)	4 (13)	3 (30)	1 (17)	0 (0)
DISCONTINUATION OF PEG-IFNA2	7 (23)	2 (20)	0 (0)	0 (0)
PBCR AT 24 MONTHS, N (%)	19 (61)	4 (40)	3 (50.0)	3 (100)
REMISSION, N (%)	11 (36)	1 (10)	4 (67)	2 (67)
BEST RELATIVE REDUCTION OF *JAK2*-V617F (%), MEAN (SD)	61 (38)	34 (25)	40 (33)	65 (33)
MOLECULAR REMISSION, N (%)	12 (39)	1 (10)	3 (50)	2 (67)

PEG-IFNα2, pegylated interferon-α2; PBCR, peripheral blood count remission

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