bjh research paper

# Ruxolitinib in elderly patients with myelofibrosis: impact of age and genotype. A multicentre study on 291 elderly patients

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## Summary

Ruxolitinib is a *JAK1/2* inhibitor that may control myelofibrosis (MF)-related splenomegaly and symptoms and can be prescribed regardless of age. While aging is known to correlate with worse prognosis, no specific analysis is available to confirm that ruxolitinib is suitable for use in older populations. A clinical database was created in 23 European Haematology Centres and retrospective data on 291 MF patients treated with ruxolitinib when aged ≥65 years were analysed in order to assess the impact of age and molecular genotype on responses, toxicities and survival. Additional mutations were evaluated by a next generation sequencing (NGS) approach in 69 patients with available peripheral blood samples at the start of ruxolitinib treatment. Compared to older (age 65–74 years) patients, elderly (≥75 years) showed comparable responses to ruxolitinib, but higher rates of drug-induced anaemia and thrombocytopenia and worse survival. Nonetheless, the ruxolitinib discontinuation rate was comparable in the two age groups. Number and types of molecular abnormalities were comparable across age groups. However, the presence of high molecular risk (HMR) mutations significantly affected survival, counterbalancing the effect of aging. Indeed, elderly patients with <2 HMR mutated genes had a comparable survival to older patients with ≥2 HMR mutations. Given that responses were not influenced by age, older age *per se* should not be a limitation for ruxolitinib administration. NGS analysis of HMR mutations also confirmed a strong predictive value in elderly patients.

**Keywords:** myelofibrosis, elderly, ruxolitinib, high molecular risk mutations, high molecular risk.


*British Journal of Haematology*, 2018, **183,** 35–46

First published online 16 July 2018
doi: 10.1111/bjh.15497

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Received 16 April 2018; accepted for publication 29 May 2018
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Myelofibrosis (MF) is the most aggressive of the Philadelphia-negative myeloproliferative neoplasms (MPNs) and may present as primary disease (PMF) or secondary to Essential Thrombocythaemia (PET-MF) or Polycythaemia Vera (PPV-MF) (Arber *et al*, 2016). The disease always follows a chronic and disabling course leading to death due to progression, disease-related or treatment-related complications, particularly thrombosis, second neoplasia and evolution to acute leukaemia. MF pathogenesis relies on mutations in three "driver" genes (namely: *JAK2, CALR, MPL*) that cause hyperactivation in the JAK-STAT pathway (Kralovics *et al*, 2005; Pikman *et al*, 2006; Nangalia *et al*, 2013). Additional "high molecular risk" (HMR) mutations in five genes (*IDH1/2, ASXL1, SRSF2, EZH2*) were identified by targeted sequencing and associated with worse outcome. (Vannucchi *et al*, 2013; Guglielmelli *et al*, 2014).

MF predominantly affects older patients, with more than 60% of diagnoses occurring in patients over 65 years of age. Specifically, the prevalence of MF in patients aged between 65 and 74 years is around 30%; however, in patients older than 75 years, it increases to over 50% (Price *et al*, 2014). Older age is a well-known risk factor for reduced survival in all MPNs and has been associated with increased incidence of leukaemic transformation. The prognosis of MF is assessed by four main scoring systems: the International Prognostic Scoring System (IPSS) (Cervantes *et al*, 2009), the dynamic-IPSS (DIPSS),(Passamonti *et al*, 2010) the DIPSS-plus (Gangat *et al*, 2011) for PMF and the Myelofibrosis Secondary to PV and ET Collaboration Prognostic model (MYSEC-PM) for PPV/PET-MF (Passamonti *et al*, 2017). Although these scores include and weigh clinical/laboratory parameters differently, older age correlates with poorer survival in all available prognostic models, and some patients may be classified at intermediate-1 or intermediate-2 risk solely or primarily because of age.

Ruxolitinib (Jakavi/Jakafi, Novartis Pharma, Origgio, Varese, Italy) is the only commercially available *JAK1/2* inhibitor that suppresses clonal myeloproliferation (*JAK2*-driven) and release of pro-inflammatory cytokines (*JAK1*-driven). In two phase III clinical studies, ruxolitinib showed proven superiority in decreasing MF-related splenomegaly and symptoms compared to placebo and best available therapy (Harrison *et al*, 2012; Verstovsek *et al*, 2012). These studies did include older patients (up to 91 years) and the median age was relatively high (66 and 67). However, no specific analysis of the elderly cohorts was performed to confirm that ruxolitinib is

suitable for use in older populations. Also, although aging is known to correlate with an increase in clonal haematopoiesis (Xie *et al*, 2014; Wahlestedt & Bryder, 2017), elderly MF patients have never been molecularly characterized.

Here, we report on the outcome of 291 patients that received ruxolitinib while older than 65 years, with particular focus on the impact of age and molecular genotype on responses, toxicities and survival. In order to further assess prognosis, next generation sequencing (NGS) analysis of 30 genes known to be involved in myeloid disorders was performed in a sub-cohort of 69 older patients with available peripheral blood samples at the start of ruxolitinib treatment.

## Patients and methods

### *Study cohort and treatment*

A recently reported multicentre observational retrospective study on World Health Organization (WHO)-defined MF was conducted in 23 European Haematology Centres (Palandri *et al*, 2017). Subjects were enrolled into the JUMP trial (NCT01493414) or treated off-study as per standard clinical practice as previously described (Palandri *et al*, 2017). All patients underwent monthly complete blood count, biochemical studies and clinic visits for 3 months and every 3 months thereafter. Data were extracted from an electronic database that included consecutive patients treated with ruxolitinib from June 2011. Data cut-off was July 2017. All treatments for MF, as well as baseline clinical/laboratory features and outcome measures (including evolution into acute leukaemia, death and spleen/symptom responses) were recorded. Diagnosis of PMF and PET/PPV-MF was made according to the WHO 2008 (Barosi *et al*, 2008; Vardiman *et al*, 2009) or the International Working Group on Myelofibrosis Research and Treatment (IWG-MRT) criteria, (Barosi *et al*, 2008) respectively. Histological examination was performed at local institutions. Sections were stained with Gomori's silver impregnation and Masson's trichrome staining to evaluate fibrosis according to the European Consensus Grading System (Thiele *et al*, 2005). Diagnosis of acute leukaemia (AL) was made according to WHO criteria, with a 20% bone marrow blast threshold for diagnosis (Arber *et al*, 2016). Ruxolitinib starting doses were administered according to prescribing information (i.e. 5 mg BID if platelet count was between 50 and 99 $\times 10^9$/l, 15 mg BID if platelet count was between 100 and 199 $\times 10^9$/l, 20 mg BID when platelet count was $\geq 200 \times 10^9$/l).

Spleen and symptoms responses as well as haematological and extra-haematological toxicities were assessed at 3, 6, 9, 12, 18 months after treatment start and at last contact during ruxolitinib therapy. All responses were defined according to 2013 IWG-MRT/European LeukaemiaNet criteria (Tefferi *et al*, 2013). Specifically, a spleen response (SR) was defined as the disappearance of splenomegaly in patients with a baseline splenomegaly palpable at 5–10 cm below the left costal margin (LCM) or as a decrease by ≥50% by palpation in case of a baseline splenomegaly palpable at >10 cm. A baseline splenomegaly palpable at <5 cm was not eligible for SR. Symptoms response required a ≥50% reduction in the 10-item Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (MPN-10) (Emanuel *et al*, 2012). Loss of response was defined as any increase in spleen size not meeting the initial response criteria. Drug-induced anaemia was defined according to National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI-CTCAE) v 4.0 (https://www.eortc.be/services/doc/ctc/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf). Patients that were transfusion-dependent before the start of ruxolitinib therapy were not evaluable for drug-related anaemia. All infections grade ≥2 according to NCI-CTCAE were recorded and included bacterial, viral and fungal episodes. Specifically, only infections that required systemic anti-infective treatment were taken into account.

The study was approved by the Institutional Review Board of each Institution and was conducted according to the Helsinki declaration.

### *Molecular and cytogenetic analysis*

Molecular tests for detection for *JAK2, MPL* and *CALR* mutations were performed as described elsewhere (Palandri *et al*, 2015).

NGS analysis was performed on DNA from granulocytes extracted from peripheral blood (PB) samples using the Myeloid Solution Kit (Sophia Genetics, Saint Sulpice, Switzerland). Amplified libraries were then sequenced on the Illumina MiSeq platform according to the manufacturer's protocol (Illumina, San Diego, CA, USA). The gene panel included entire or specific exons of 30 genes known to be involved in myeloid disorders (*ABL1, ASXL1, BRAF, CALR, CBL, CEBPA, CSF3R, DNMT3A, ETV6, EZH2, FLT3, IDH1, IDH2, HRAS, KIT, KRAS, JAK2, MPL, NPM1, NRAS, PTPN11, RUNX1, SETBP1, SF3B1, SRSF2,TET2, TP53, U2AF1, ZRSR2, WT1*). Variants – including single nucleotide polymorphism (SNP) and insertions/deletions (indels) – if not well covered, synonymous, inframe, in untranslated regions (UTRs) or in introns were excluded from the analysis. Variants were pre-classified as potentially pathogenic by SOPHiA™ Artificial Intelligence (Sophia Genetics). Output data was analysed by Sophia DDM v3 software (Sophia Genetics). High molecular risk (HMR) pathogenetic mutations were defined as those including *ASXL1, SRSF2, EZH2, IDH1* and *IDH2*. Only variants (single mutations and indels) that were potentially damaging at functional level with a critical impact on disease pathogenesis and progression were considered for the present analysis.

Chromosome banding analysis was performed on marrow cells by standard banding techniques according to the

International System for Human Cytogenetic Nomenclature (Shaffer *et al*, 2009).

### *Statistical analysis*

Continuous variables were expressed as median and ranges; categorical variables were presented as frequencies and percentages. Comparisons between groups were carried out by Wilcoxon-Mann-Whitney rank-sum test or *t* test, and association between categorical variables (2-way tables) was tested by the Fisher exact test or $\chi^2$, as appropriate. Where variables were ordinal, the test for trend developed by Cuzick was performed.

Survival analysis was performed by means of Kaplan–Meier (KM) curves and differences between KM curves were evaluated using the Log-rank test. Specifically, overall survival (OS) was calculated from the date of ruxolitinib start to the date of death or to last contact, whichever came first. Progression-free survival (PFS) included death and leukaemic transformation. Combined event-free survival (EFS) comprised death, ruxolitinib discontinuation from any cause, and progression to acute leukaemia. Cumulative incidences of infections, ruxolitinib discontinuation and evolution into acute leukaemia were calculated considering death as competing risk, according to the model of Fine and Gray. All tests were 2-sided and a *P* values less than 0·05 were considered statistically significant. Analyses were performed with STATA software v.15 (StataCorp LLC, College Station, TX, USA).

## Results

### *Study cohort*

Between June 2011 and April 2016, 462 patients with PMF (*n* = 249, 53·9%), PET-MF (*n* = 84, 18·2%) or PPV-MF (*n* = 129, 27·9%) were treated with ruxolitinib in 23 European Haematology Centres.

Two hundred and ninety-one (63%) patients started ruxolitinib when aged 65 years or older and were included in the present analysis. Overall, 126 patients on study received ruxolitinib as compassionate or commercial use; 165 (56·7%) patients were first enrolled in the JUMP trial which was closed for enrolment in September 2014. At MF diagnosis, median age was 69·4 years (range, 38–88·1). Anaemia (haemoglobin <100 g/l) was present in 91 (32·3%) patients. Splenomegaly was appreciable ≥10 cm below LCM in 144 (49·5%) patients; 144 patients had constitutional symptoms.

At ruxolitinib start, median age was 73·1 years (range, 65·1–89·0); median haemoglobin was 103 g/l (range, 50–167) and 90 (30·9%) patients had a transfusion-dependent anaemia. Median platelet count was 249 × $10^9$/l (range, 32·9–1887). Most (96·6%) patients had spleen enlargement (spleen length was palpable ≥10 cm below LCM in 67%). Median total symptoms score (TSS) was 20 (0–100). IPSS risk was intermediate-1 (16 patients, 5·5%), intermediate-2 (138 patients, 47·4%) or high (137 patients, 47·1%). Marrow fibrosis was evaluable in 268 (92·1%) patients and was grade 1 in 68 (25·4%), grade 2 in 115 (42·9%), and grade 3 in 80 (29·8%) patients. Karyotype was abnormal in 40 (27·2%) out of 147 evaluable patients. In 9 cases (6·6%) an unfavourable karyotype was detected, specifically: trisomy 8 (2 patients), complex (4 patients), del7 (2 patients) and del5 (1 patient). Median follow-up from MF diagnosis was 3·9 years (range, 0·17–35·27) and median ruxolitinib exposure was 17·4 months (range, 0·9–67·2). Patients were stratified according to age at ruxolitinib start (older: age 65–74 years; elderly; age ≥75 years). The two cohorts of patients had comparable baseline characteristics in terms of large splenomegaly, symptoms burden and transfusion-dependent anaemia. Nonetheless, elderly patients were more frequently classified as high IPSS risk and had a significantly lower baseline platelet count, resulting in lower ruxolitinib starting doses. Ruxolitinib starting dose was decided accordingly to prescribing information in 79·7% of the patients, without significant differences in the two age groups ($P = 0·49$). In the remaining 59 patients, the starting ruxolitinib dose was lower compared to standard indications.

Dose reductions during the first 12 weeks of therapy were required in 34·7% of patients and were comparable in older and elderly patients ($P = 0·18$).

Overall, 45 out of 291 patients did not undergo a molecular evaluation (15·5%); 11 (3·8%) were *JAK2*$^{V617F}$-negative but were not assessed for *CALR/MPL*. Among the remaining 235 (80·7%) patients, *JAK2*$^{V617F}$ was present in 200 (85·1%) patients, *CALR* mutations in 24 (10·2%) and *MPL*$^{W515K/L}$ in 2 (0·9%); 9 (3·8%) of the patients were triple negatives. Median *JAK2*$^{V617F}$ allele burden was 45% (range, 2%–99%), with 89 (44·5%) *JAK2*$^{V617F}$-positive patients being homozygous. The proportion of patients with the *JAK2*$^{V617F}$ mutation and mutation load was comparable in the two age groups (Table I).

### *Impact of age on response to treatment*

Spleen response (SR) was evaluable in 253 out of 291 (86·9%) patients. A total of 125 (49·4%) patients with spleen ≥5 cm achieved SR at least in one evaluation over the first 3 years from ruxolitinib start. At 3 and 6 months, the response was achieved by 25·7% and 35% of 253 and 220 evaluable patients, respectively. The overall SR rate was comparable in the two age groups (49·7% in older patients vs. 49% in the elderly, $P = 0·92$). Analogously, older age did not influence SR at 3 and 6 months (25·5% and 26% at 3 months in older patients and elderly, $P = 0·93$; 34·8% vs. 35·2% at 6 months, $P = 0·95$).

Systemic symptoms were sequentially evaluated in 272 patients who completed the MPN-SAF TSS. Overall, 233 (85·3%) achieved a symptom response (SyR, defined as ≥50% reduction in the MPN-SAF TSS) by 3 years from

**Table I.** Patients characteristics according to age at ruxolitinib start.

| Characteristics | Age 65–74 years ($n$ = 179) | Age ≥75 years ($n$ = 112) | $P$ value |
|---|---|---|---|
| Male sex, $n$ (%) | 109 (60·89%) | 65 (58·04%) | 0·63 |
| Primary MF, $n$ (%) | 95 (53·1%) | 61 (54·46%) | 0·82 |
| *JAK2*$^{V617F}$ mutation, $n$ (% on 235 evaluable) | 122 (82·4%) | 78 (89·7%) | 0·17 |
| *JAK2*$^{V617F}$ mutation load ≥50%, $n$ (% on 154 evaluable) | 53 (55·2%) | 36 (62·1%) | 0·40 |
| IPSS, $n$. (%) | | | |
| Intermediate-1 | 13 (7·3%) | 3 (2·7%) | <0·001 |
| Intermediate-2 | 94 (52·5%) | 44 (39·3%) | |
| High | 72 (40·2%) | 65 (58%) | |
| Median haemoglobin, g/l (range) | 105 (68–158) | 99 (50–167) | 0·40 |
| Haemoglobin <100 g/l | 77 (43·0%) | 58 (51·8%) | 0·14 |
| Transfusion dependence, $n$ (%) | 51 (28·5%) | 39 (34·8%) | 0·26 |
| Median platelet count, $\times 10^9$/l (range) | 281 (52–1632) | 213 (32·9–1887) | <0·001 |
| Platelet count ≥200 $\times$ $10^9$/l | 122 (68·2%) | 58 (51·8%) | 0·005 |
| Platelet count <100 $\times$ $10^9$/l | 11 (6·1%) | 19 (17·0%) | 0·003 |
| Constitutional symptoms, $n$ (%) | 81 (45·5%) | 63 (57·3%) | 0·05 |
| Palpable spleen, $n$ (%) | 170 (95%) | 111 (99·1%) | 0·06 |
| Spleen ≥10 cm, $n$ (%) | 121 (67·6%) | 74 (66·1%) | 0·79 |
| Unfavourable karyotype, $n$ (% on 147 evaluable) | 8 (8·3%) | 1 (2·0%) | 0·12 |
| Median CCI (range) | 1 (0–8) | 1 (0–8) | 0·16 |
| CCI ≥2, no (% on 248 evaluable) | 65 (41·7%) | 45 (48·9%) | 0·27 |
| Median body mass index (range) | 23·9 (16·7–33·3) | 23·8 (15·6–31·2) | 0·10 |
| Marrow fibrosis grade, no (% on evaluable*) | | | |
| Grade 1 | 44 (26·7%) | 24 (23·3%) | 0·22 |
| Grade 2 | 75 (45·4%) | 40 (38·8%) | |
| Grade 3 | 42 (25·4%) | 38 (36·9%) | |
| Time from MF diagnosis to ruxolitinib start >2 years | 78 (43·6%) | 45 (40·2%) | 0·81 |
| Ruxolitinib starting dose | | | |
| 5 mg BID | 17 (9·5%) | 23 (20·5%) | 0·02 |
| 10 mg BID | 20 (11·7%) | 8 (7·1%) | |
| 15 mg BID | 42 (23·5%) | 29 (25·9%) | |
| 20 mg BID | 100 (55·9%) | 52 (46·4%) | |
| Ruxolitinib 12-week titrated dose | | | |
| 5 mg BID | 36 (20·6%) | 35 (31·8%) | 0·06 |
| 10 mg BID | 35 (20·0%) | 15 (13·6%) | |
| 15 mg BID | 51 (29·1%) | 25 (22·7%) | |
| 20 mg BID | 53 (30·3%) | 35 (31·8%) | |
| Median follow-up from ruxolitinib start (months) | 22·9 (1·1–67·2) | 14·3 (0·5–56·7) | 0·01 |

CCI, Charlson Comorbidity Index; MF, myelofibrosis.

*Fibrosis grade was evaluable in 165 older patients and 103 elderly patients. Grade 1 fibrosis was present in 68 of 156 patients with Primary Myelofibrosis.

therapy start. The overall SyR rate was comparable in older and elderly patients (85·4% vs. 86·1%, $P$ = 0·86). At 3 and 6 months, 208 out of 271 (76·8%) and 185 out of 221 (83·7%) evaluable patients achieved SyR. These were comparable in older and elderly patients at both time-points (78·0% and 74·8% at 3 months in elderly and very elderly, $P$ = 0·53; 85·7% vs. 80·7% at 6 months, $P$ = 0·32).

### *Impact of age on ruxolitinib-related toxicity*

Ninety (30·9%) patients were transfusion-dependent before the start of ruxolitinib therapy and were considered not evaluable for drug-related anaemia.

Overall, 180 out of 193 (93·3%) evaluable patients developed anaemia of any grade during ruxolitinib therapy (Table II). The proportion of patients with any grade of anaemia during ruxolitinib therapy was higher in elderly patients (98·6% vs. 90·2% $P$ = 0·04). However, the proportion of patients acquiring transfusion-dependent anaemia was similar in the two groups (31·7% vs. 38·6% in the elderly, $P$ = 0·33). At 3 and 6 months, anaemia of any grade occurred in 174 and in 137 out of 193 (90·1%) and 171 (80·1%) evaluable patients, respectively. The proportion of patients with anaemia at 3 and 6 months was not influenced by age.

Overall, 158 out of 283 evaluable patients (55·8%) had a thrombocytopenia of any grade during treatment, with a

**Table II.** Haematological toxicity during ruxolitinib therapy according to age at treatment start.

| | Anaemia | | | Thrombocytopenia | | |
|---|---|---|---|---|---|---|
| | Age 65–74 years | Age ≥75 years | *P* value | Age 65–74 years | Age ≥75 years | *P* value |
| Any time, all grades | 90·2% | 98·6% | 0·04 | 51·1% | 63·3% | 0·04 |
| At 3 months, all grades | 87% | 95·7% | 0·07 | 41% | 54·1% | 0·03 |
| Grade 3/4 | 30% | 34·3% | 0·63 | 4% | 6·4% | 0·37 |
| At 6 months, all grades | 76·4% | 86·9% | 0·10 | 43·7% | 52·2% | 0·21 |
| Grade 3/4 | 25·4% | 31·1% | 0·42 | 2·1% | 4·3% | 0·31 |

Percentages were calculated on the number of evaluable patients at each time-point.

higher incidence in the elderly ($P = 0·04$). At 3 months, thrombocytopenia occurred in 130 out of 282 (46·1%) evaluable patients and was more frequent in the elderly ($P = 0·03$). Similarly, 111 out of 236 (47%) patients had a thrombocytopenia of any grade at 6 months, with increased incidence in the elderly. However, overall grade 3–4 thrombocytopenia was comparable in the two groups.

After a median follow-up of 19·5 months from ruxolitinib start, 92 out of 282 (32·6%) evaluable patients developed a total of 116 grade ≥2 infectious events, for an incidence rate of 23·2 per 100 patients-years. Using death without infection as competing risk, the cumulative incidence of infection was 17%, 24·2% and 31·1% at 6, 12 and 24 months, respectively, and was not significantly influenced by older age ($P = 0·77$).

### *Impact of age on Ruxolitinib discontinuation and outcome*

Overall, 116 (39·9%) patients discontinued ruxolitinib after a median drug exposure of 12·5 months (range, 0·9–39·3). Causes of discontinuations were: disease progression (22·1%); acute leukaemia (14·2%); lack of response (13·3%); infections (11·5%); drug-related haematological toxicity (9·7%); heart disease (7·1%); bleeding (3·5%); second neoplasia (1·8%); allogeneic transplant (0·9%) and other unrelated causes (15·9%). Notably, patients that discontinued due to thrombocytopenia (platelet count $<100 \times 10^9$/l) or clonal evolution had a significantly lower survival compared to patients that discontinued for other reasons (*log-rank* $P = 003$ and $P = 0·01$, respectively).

The percentage of patients discontinuing ruxolitinib was higher in the elderly cohort (35·2% vs. 47·3%, $P = 0·04$). However, the cumulative incidence of ruxolitinib discontinuation was comparable in older and elderly patients, after adjusting for the risk of death ($P = 0·77$).

A total of 23 patients developed acute leukaemia, after a median time from ruxolitinib start of 16·3 months (range, 3·2–39·6). The cumulative incidence of progression to acute leukaemia, considering death as a competing risk ($P = 0·85$), and the leukaemia-free survival (LFS) (*log-rank* $P = 0·54$) were comparable in older and elderly patients ($P = 0·85$ and $P = 0·54$, respectively). LFS at 12 months was 96·3% and 91·3% in the two cohorts, respectively.

Eighty-five (29·2%) patients died after a median time from ruxolitinib start of 15·4 months (range 1·5–56·7). Causes of death were, specifically: progression of myelofibrosis (40%), evolution into AL (16·5%), infections (14·1%), heart disease (10·6%), thrombotic/haemorrhagic events (7·1%), allogeneic transplantation (1·2%), second neoplasia (1·2%) and other unrelated causes (9·4%).

As expected, the elderly patients showed a significantly worse survival compared to the older patients (*log-rank* $P < 0·001$). Analogously, the 12-month PFS and combined EFS were significantly worse in the elderly (*log-rank* $P < 0·001$ and $P = 0·02$, respectively) (Fig 1). The same results were confirmed by relative survival analyses taking into account only MF-related deaths (data not shown).

Notably, responses and toxicity rates, as well as OS, were comparable in larger centres, including more than 20 patients, and smaller centres with less than 20 patients enrolled (Table SI).

### *Molecular genotype by NGS analysis according to age*

Overall, 69 out of 291 patients (23·7%) had DNA available for NGS screening before ruxolitinib start. Clinical and laboratory features of these patients were similar to those observed in patients with no PB samples available (Table SII). Median age of the 69 evaluable patients was 72·5 years (range 65·2–83·1). There were 32 (46·4%) cases of PMF, 23 (33·3%) cases of PPV-MF and 14 (20·3%) cases of PET-MF.

A total of 193 variants, including 143 SNP and 50 indels, were identified with a mean of 2·8 variants per patient (range 0–6). Twenty-one out of 30 genes were mutated in at least one patient, while 9 genes (*BRAF, FLT3, HRAS, WT1, KIT, SETBP1, ABL1, CBL, NPM1*) were never mutated. Overall, 68 (98·6%) patients had at least one variant in ≥1 gene (Fig 2). Specifically, 13 (18·8%) patients harboured one, 23 (33·3%) patients two, 17 (24·6%) patients three, 10 (14·5%) patients four and 5 (7·2%) patients five mutated genes. The most recurrently mutated genes were: *ASXL1* (mutated in 43·5% of patients), *TET2* (34·8%), *DNMT3A* (10·1%) and *SRSF2* (10·1%).

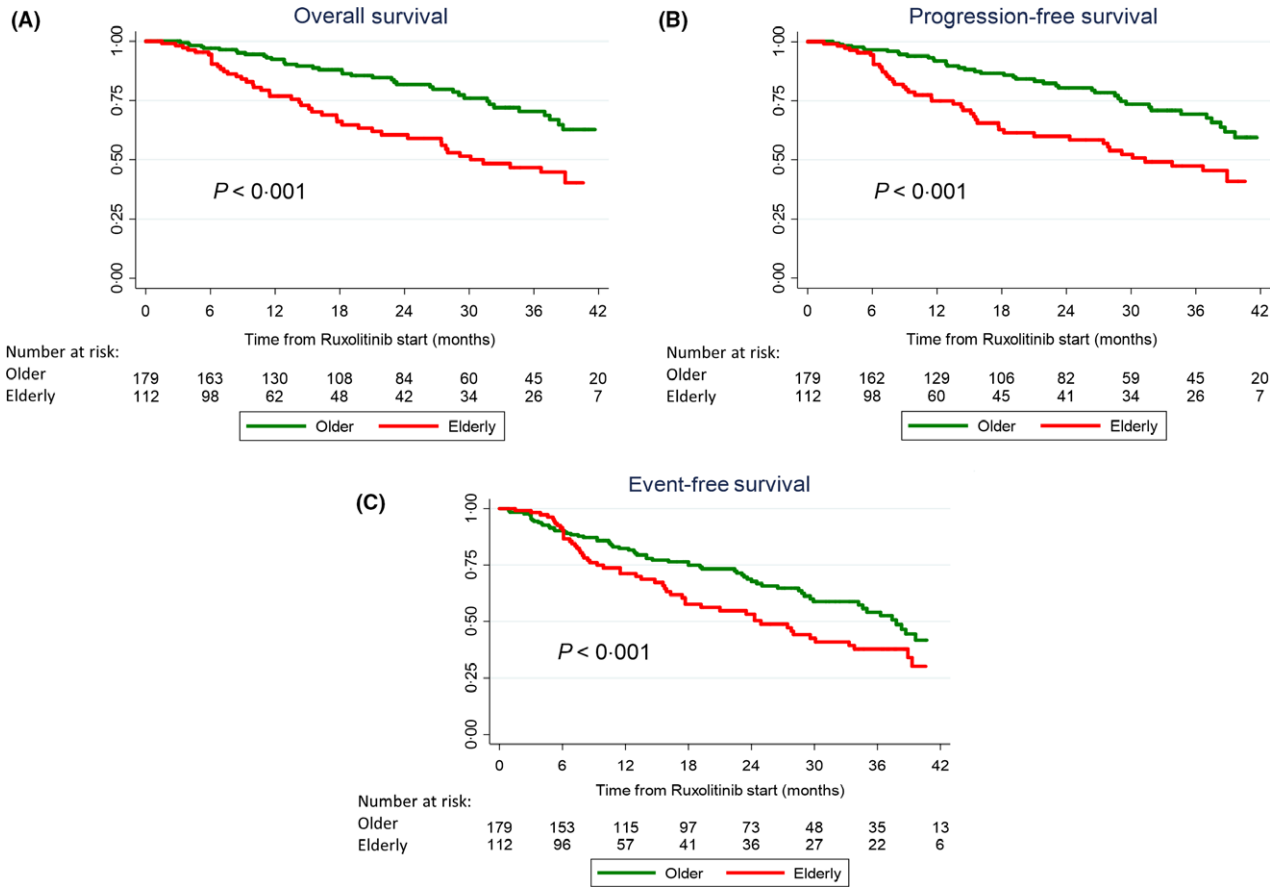


**Fig 1.** Outcome parameters by age. Older (age 65–74 years) patients had a significantly better survival compared to elderly patients (age ≥75 years). [Colour figure can be viewed at wileyonlinelibrary.com]

Driver mutations distribution was: *JAK2*$^{V617F}$ (54 patients, 78·3%), *CALR* (12 patients, 17·4%), *MPL*$^{W515L}$ (1 patient, 1·4%); 2 patients were triple negative (TN). Ten out of 54 (18·5%) patients with the *JAK2*$^{V617F}$ mutation showed a single *JAK2* mutation while the remaining 44 cases showed additional mutations in *TET2, ASXL1, SRSF2, DNMT3A, EZH2, U2AF1, RUNX1, ZRSR2, TP53, SF3B1, CSFR3R, CEBPA, IDH2, MPL, IDH1, ETV6, NRAF* and *PTPN11* (in descending order). Of the 12 patients with *CALR* mutation, one (8·3%) patient showed a single *CALR* mutation while the remaining 11 patients showed additional mutations in *ASXL1, TET2, DNMT3A, TP53, JAK2, EZH2, SRSF2, NRAS, RUNX1, SF3B1, KRAS* (in descending order). The *MPL*$^{W515L}$ mutation was a single molecular abnormality.

At least one HMR mutation was present in 52·2% of patients; 11·6% had ≥2 HMR mutations. *ASXL1* mutations were principally associated with *JAK2* (20 cases), *TET2* (10 cases), *CALR* (10 cases), *EZH2* (4 cases), *TP53* (4 cases); *SRSF2* with *JAK2* (6 cases), *ASXL1* (3 cases), *TET2* (3 cases), *TP53* (2 cases); *EZH2* with *JAK2* (4 cases), *ASXL1* (4 cases), *RUNX1* (2 cases); *IDH1/2* with *JAK2* (3 cases), *TET2* (2 cases) and *SRSF2* (1 case).

Forty-nine (71%) of the 69 patients analysed by targeted sequencing were older patients (age 65–74 years) and 20 were elderly (age ≥75 years). The number of variants and mutated genes were comparable in the two age groups ($P$ = 0·21 and $P$ = 0·17, respectively). Additionally, driver mutations distribution was comparable in the two age groups. Nonetheless, *TP53* and *RUNX1* were more frequently mutated in the elderly (20% vs. 2%, $P$ = 0·009; 15% vs. 2%, $P$ = 0·04, respectively).

Karyotype was available in only 50·5% of patients, mostly due to dry tap. Abnormal karyotype (17 patients) was not significantly associated with specific mutations.

### *Impact of molecular genotype on outcome according to age*

Molecular status was investigated for association with outcome parameters. No significant difference in survival ($P$ = 0·64) was observed when comparing patients with only *JAK2* mutation and patients with *JAK2* combined with additional mutations. Conversely, patients with ≥4 mutated genes showed a significantly worse OS ($P$ = 0·02) and PFS compared to patients with a lower number of mutated genes ($P$ = 0·04). Of note, LFS was significantly shorter in patients carrying leukaemic transformation-related *TP53* and *RUNX1* mutations ($P$ = 0·01).

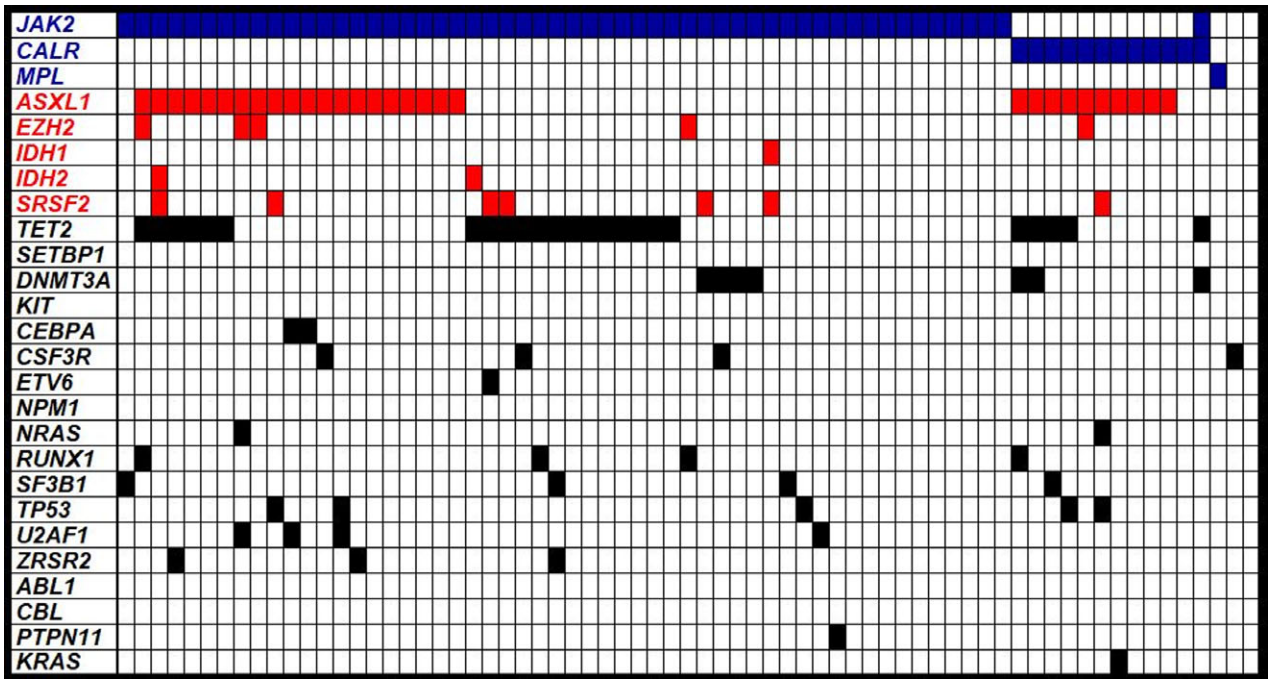


**Fig 2.** Plot of mutations by case. All *JAK2* mutations were V617F except for one. [Colour figure can be viewed at wileyonlinelibrary.com]

Additionally, not only the number but also the type of variants is relevant in MF, as demonstrated by prognostic detrimental mutations in high-molecular risk (HMR) genes, such as *ASXL1*, *EZH2*, *IDH1/2* (Guglielmelli *et al*, 2014). Indeed, the presence of at least 2 HMR mutated genes was significantly associated with worse OS ($P = 0{\cdot}005$), LFS ($P = 0{\cdot}003$), PFS ($P = 0{\cdot}009$) and EFS ($P < 0{\cdot}001$). Of note, the presence of <2 HMR mutations could counterbalance the negative effect of older age on survival. Indeed, elderly patients with <2 HMR mutated genes had a comparable survival to older patients with ≥2 HMR mutated genes (Fig 3).

As a result, the two age-groups, if stratified according to the number of HMR variants (positive if ≥2, otherwise negative) were distributed in 3 new risk-categories: (i) elderly HMR-positive patients, who had the worst survival; (ii) older HMR-negative patients, who had the best outcome and (iii) elderly HMR-negative patients and older HMR-positive patients, classed as patients with intermediate survival.

## Discussion

The introduction of tyrosine kinase inhibitors was associated with improved outcomes in older patients with Philadelphia-positive chronic myeloid leukaemia (CML) (Breccia *et al*, 2012). Here, we report efficacy and safety data on the use of ruxolitinib in a cohort of older and elderly patients homogeneously treated with the tyrosine kinase inhibitor, ruxolitinib.

The first result of the study is that elderly patients treated with ruxolitinib could achieve therapeutic results comparable to younger patients, both in terms of spleen and symptoms responses. While infectious rates were not increased by age, elderly patients had a slightly higher incidence of all-grade anaemia and thrombocytopenia, probably due to a lower baseline platelet count, which resulted in significantly lower ruxolitinib starting doses and a trend to lower 12-week titrated doses. However, the incidence of severe (grade 3–4) and transfusion-dependent anaemia was comparable across both age groups. The fact that ruxolitinib was comparably discontinued in both age groups shows how the elderly could maintain the treatment over time, analogously to younger patients. Additionally, our study confirms a previous report showing that the reason for ruxolitinib discontinuation may affect prognosis, with patients that discontinued due to thrombocytopenia or clonal evolution having the worse outcome (Newberry *et al*, 2017).

The second result is that aging, despite not influencing the efficacy and safety of ruxolitinib, had a significant impact on outcome, in terms of worse OS, PFS and EFS. This result, which was to some extent expected, may not be attributed to excess in comorbidities in the elderly that at ruxolitinib start had a Charlson Comorbidity Index comparable to the older patients, but confirm the pivotal role of aging in all outcome parameters.

The clinical study was further enriched by an NGS analysis performed in a sub-cohort of 69 patients aged ≥65 years, including 20 elderly (≥75 years) cases. Here, we identified mutations in 21/30 genes frequently mutated in haematological malignancies, with 18 non-driver mutations in at least one patient. Conversely, nine genes, including those encoding transcription factors (*FLT3, CBL, WT1*), cytokine receptors (*KIT*), protein kinases (*BRAF, ABL1*), enzymes (*HRAS*) and oncogenes (*SETBP1, NPM1*), were never mutated in our ≥65-year-old MF patients, ruling out their role in disease onset.

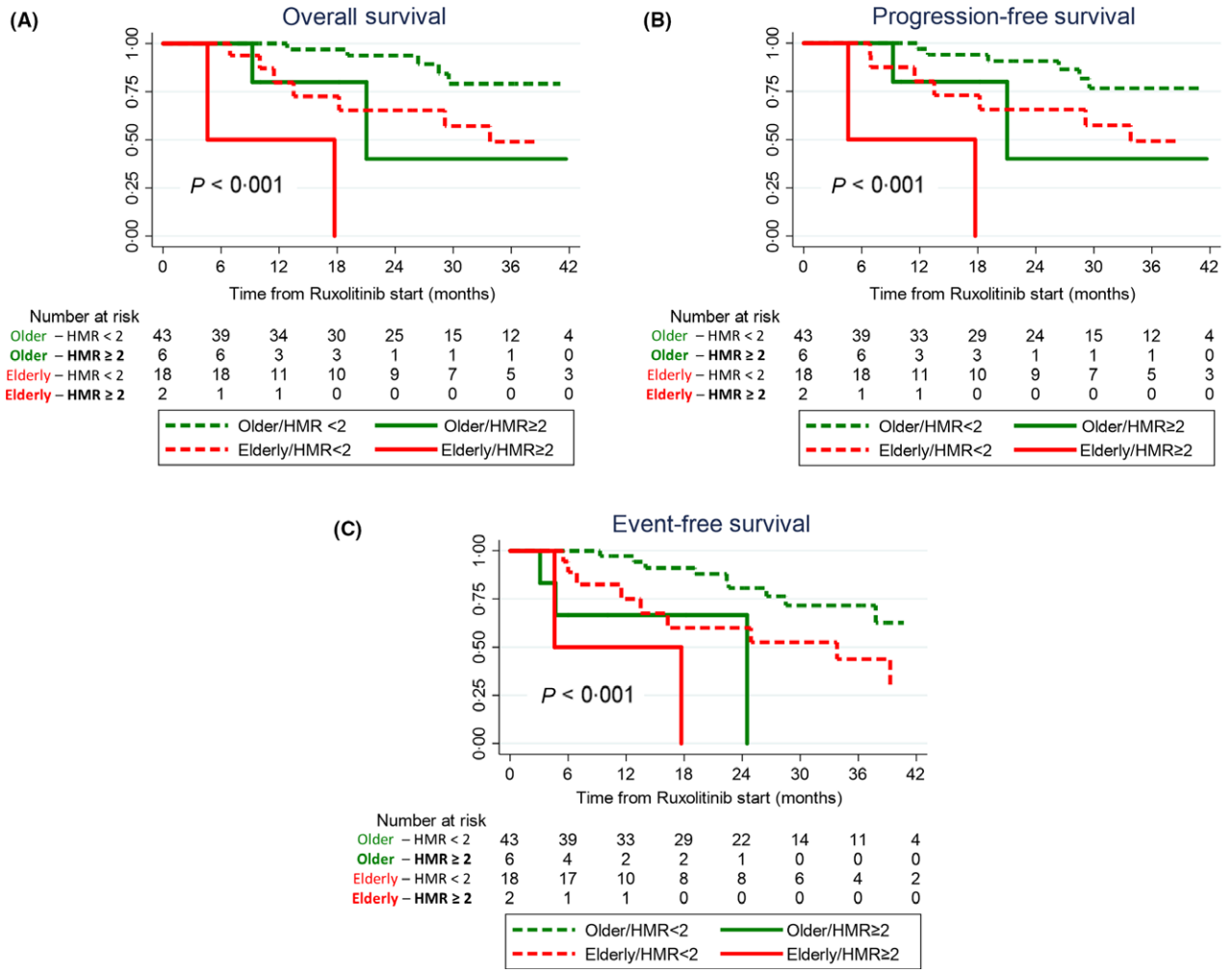


**Fig 3.** Overall survival (A), progression-free survival (B) and event-free survival (C) according to HMR and age. Elderly and older patients at high molecular risk (HMR) had a median overall survival of 4·6 and 21·1 months, respectively, while elderly and older patients with <2 HMR had a median overall survival of 33·8 months and not reached, respectively. [Colour figure can be viewed at wileyonlinelibrary.com]

Moreover, all patients but one had a non-driver mutation and 56 (81%) patients harboured mutations in two or more genes. The mutational frequency of *JAK2*, *CALR* and *MPL* was in line with literature findings (Barbui *et al*, 2018). The most recurrently (co)mutated genes were those that encoded epigenetic (*ASXL1*, *TET2*, *DNMT3A*) and splicing regulators (*SRSF2*). Mutations of both *TET2* and *DNMT3A* (which are principally involved in DNA methylation) increase the self-renewal capacities of haemopoietic stem cells in both humans and mice and play an important role in disease initiation; moreover, they are the two most frequently mutated genes associated with clonal haemopoiesis during aging (Vainchenker & Kralovics, 2017). It can therefore be hypothesized these mutations may favour the occurrence of secondary mutations by inducing clonal haemopoiesis and supporting the replication of mutated cells.

Overall, we could not detect a significant difference in the genetic landscape between our older/elderly patients, suggesting that molecular complexity is not significantly increased by age but is mostly MF-driven. Nonetheless, elderly patients more frequently carried leukaemic-transformation related mutations in *TP53* and *RUNX1* (Vainchenker & Kralovics, 2017), which also correlated with a shorter LFS, indicating that aging may promote the occurrence of a leukaemic-promoting molecular landscape in patients with MF. A higher number of variants as well as the presence of high-risk mutations inversely correlated with survival, supporting that the study of clonal complexity is of prognostic relevance. Of note, while $JAK2^{V617F}$ mutations were principally associated with mutations in *ASXL1, SRSF2, EZH2* and *IDH1/2*, *CALR* mutations were associated with *ASXL1* mutations only, confirming that biological differences between *JAK2* and *CALR* mutated patients are also influenced by the quantity and types of co-mutated HMR genes. (Guglielmelli *et al*, 2017).

Notably, we found that HMR status (≥2 HMR mutated genes) significantly impacted on outcome, irrespective of age. Surprisingly, elderly patients with <2 HMR mutated genes displayed a survival probability similar to the older patients

with ≥2 HMR mutated genes, while elderly patients with ≥2 HMR had the worst survival. In conclusion, MF patients (under ruxolitinib treatment and ranging from 65 to 89 years old) are not indistinct entities but, thanks to a multigene sequencing approach, can be characterised in 3 prognostic classes of survival. Despite the limitations of the NGS subcohort size, our results indicate that a favourable HMR molecular status may overcome the negative impact of older age and that mutations – more than aging – may have a driver/determinant effect on patient survival. Overall, NGS study of HMR mutations also was confirmed to be relevant for assessing prognosis in the over 65-year-old population that is not eligible for allogeneic stem cell transplantation. Accordingly, all prognostic scores recently proposed for MF also include evaluation of HMR mutations (Guglielmelli *et al*, 2018).

We acknowledge that patients were included in this specific retrospective analysis only if they started ruxolitinib therapy when aged ≥65 years and this represents a selection. However, the therapeutic decision to start ruxolitinib was not based on age, but on clinical needs that were evaluated on a case-by-case basis by treating physicians. We also acknowledge that in our cohort the presence of ≥2 HMR mutations significantly affected outcome, whereas one HMR mutation was not associated with worse survival. It is likely that this result is related to the low number of patients that were evaluated for HMR status and by the relatively high proportion of patients carrying at least 1 HMR mutation (52·2%). However, the possibility that these results may reflect a specific feature of this peculiar patient population, which has never been analysed before, cannot be excluded. Further studies in larger cohorts may help in driving more definitive conclusions on this issue.

Overall, the results from this large cohort of patients show that no upper age limit should be applied for the administration of ruxolitinib to patients with MF; nonetheless, a slight increase in overall haematological toxicity may be expected. Additionally, the presence of HMR mutations was found to overcome the effect of aging, confirming a strong prognostic role also in the elderly population.

## Acknowledgements

We thank Dr Maria Letizia Bacchi Reggiani for her contribute in the statistical revision. We also thank Dr Matteo Renzulli for his support in the NGS analysis.

## Authors' contribution

FP, LC, MRS, RL: coordinated the study and wrote the paper; DB performed the statistical analysis; LC, MRS, EO: performed molecular analysis. All Authors: designed the study and collected clinical, laboratory, molecular and histology data. All Authors gave final approval to the manuscript.

## Declaration of interests

F.P., M.Ti. and M.Tr. acted as consultant and received honoraria from Novartis; G.A.P. honoraria from Novartis, Celgene, Janssen, Amgen, Hospira, Teva; G.Be. honoraria from Novartis, Janssen, Amgen; A.Iu., M.Br., E.A., M.Bo. and N.S. honoraria by Novartis, BMS, Pfizer, Incyte; A.Is. honoraria from Novartis, Gilead, Janssen; G.S. honoraria from Abbvie, Roche, Takeda; M.Cr. honoraria form Janssen, Novartis, BMS, Celgene; F.C. honoraria from Novartis, Incyte, Pfizer; R.L. honoraria from Novartis, Celgene, BMS, Janssen; F.A. honoraria from Gilead, Sigma-Tau, Astellas, Roche; R.M.L. honoraria from Gilead, Novartis, Sanofi, Milteny.

## Funding source

The paper was funded by BolognAIL, AIRC and the European project Harmony-IMI2.

## Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table SI.** Comparison of responses and toxicity rates according to the number of patients included by different Hematology Centers. Centers were categorized in two groups according to the number of included patients (<20 patients: 17 Centers, total of 136 patients included; ≥20 patients: 6 Centers, total of 155 patients included). Median follow-up time was longer for patients enrolled in larger Centers (24.2 months vs. 16.4 months, $P = 0.01$). Percentages were calculated on intention-to-treat analysis.

**Table SII.** Patients characteristics according to evaluation by next generation sequencing (NGS). Patients were selected for NGS analysis only on the base of availability of DNA for NGS screening before ruxolitinib start.

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