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Ropeginterferon-alfa2b resolves angina pectoris and reduces *JAK2V617F* in a patient with clonal hematopoiesis of indeterminate potential: A case report

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The *JAK2V617F* mutation is an acquired somatic mutation, which is prevalent in patients with the Philadelphia-chromosome negative myeloproliferative neoplasms (MPNs). In these diseases the mutation gives rise to constitutive JAK-STAT signaling with increased blood cell counts and *in vivo* activation of neutrophils and platelets as well, which altogether contribute to a chronic inflammatory and thrombogenic state with a 12-fold increased risk of coronary disease. Treatment with recombinant interferon-alpha2 (rIFN) reduces the *JAK2V617F* allelic burden in a large number of MPN-patients. Long-term treatment with rIFN associates with low-burden *JAK2V617F* in a subset of patients and a decreased thrombosis risk as well. In the general population the *JAK2V617F* mutation has been shown to associate with ischemic heart disease and thrombosis. Based upon the above observations we herein report the first patient with CHIP-*JAK2V617F*, in whom treatment with rIFN resolved severe angina pectoris. During a short period off rIFN the symptoms reappeared to resolve in concert with reduction of *JAK2V617F* allele burden, when rIFN was reinstituted. The *JAK2V617F* mutation may be a novel therapeutic target to prohibit the development of cardiovascular diseases using rIFN either as monotherapy or in combination with potent anti-inflammatory agents.

KEYWORDS

***JAK2V617F* mutation, clonal hematopoiesis of indeterminate potential (CHIP), angina pectoris, recombinant interferon-alpha2, myeloproliferative neoplasms**

Highlights

- Complete resolution of angina pectoris and major molecular remission in a CHIP-*JAK2V617F* patient with severe ischemic heart disease.
- In cardiovascular disease CHIP-*JAK2V617F* positive patients, the mutation may be a novel molecular target for rIFN to be investigated in future trials.

Introduction

The *JAK2V617F* mutation is prevalent in the Philadelphia-chromosome negative myeloproliferative neoplasms (MPNs) (1) and associates with a 12-fold increased risk of coronary disease (2, 3), which may be explained by its potential to induce a chronic inflammatory state by generating reactive oxygen species (4). Furthermore, this mutation also induces increased Peptidyl Arginine Deiminase Type 4 (PAD4), which is required for the formation of *JAK2V617F*-driven Neutrophil Extracellular Traps (NETs) and thrombosis (5). Other thrombosis-promoting mechanisms are elevated blood cell counts and their constitutive *in vivo* activation with formation of circulating microaggregates and impaired microcirculation (2). The above thrombogenic factors contribute significantly to morbidity and mortality in MPNs (2, 3). Taking into account the detrimental effects of the *JAK2V617F* mutation (2, 3, 6, 7), it is a great advantage that treatment with pegylated interferon alpha2 (rIFN) is able to reduce the *JAK2V617F* allelic burden (8–17) and in a subset of patients even to very low levels (< 1%), inducing minimal residual disease (MRD) (13–15). Thus, last year ropegInterferon (ropegIFN) was launched for the treatment of patients with PV (16, 17).

The development of MPNs is for decades preceded by Clonal Hematopoiesis of Indeterminate Potential (CHIP) which increases with ageing and associates with an increased risk of cardiovascular disease (CVD) (18). The *JAK2V617F* mutation is far more prevalent in the general population than previously anticipated with a prevalence of approximately 5% in individuals above 60 years (19). Importantly, *JAK2V617F*-positive clonal hematopoiesis associates with an increased incidence of thrombosis in the general population without a known myeloid disorder (5, 19).

Mathematical modelling studies have shown that the earlier treatment with rIFN is instituted in MPNs the better, since the *JAK2V617F* mutation will then more rapidly decline during treatment (20). Importantly, the *JAK2V617F* mutation might actually be a novel therapeutic target in the CHIP-stage, thereby potentially eradicating the malignant clone and prohibiting development of MPNs and associated comorbidities, including CVD (13, 21). Herein, we for the first time report the complete resolution of angina pectoris in a male patient with CHIP-*JAK2V617F* during treatment with rIFN.

Case story

A 50-year-old male patient was admitted with attacks of angina pectoris. His history included hypertension, type 2 diabetes mellitus, hypercholesterolemia and coronary sclerosis. In June 2019 the patient suffered myocardial infarction. In December 2019, an angiography displayed in-stent re-stenosis of previously applied stents and *de-novo* stenosis of other coronary segments. Three new stent implantations were performed. However, heart complaints persisted with daily, frequent angina pectoris, despite optimal medication for CVD, including aspirin and atorvastatin.

In January 2021 frequent attacks of angina appeared. A coronary angiography necessitated new stent implantation and a drug eluting balloon angioplasty. Despite these procedures, daily cardiac angina complaints persisted from March of 2021.

From January 2021 blood cell counts were repeatedly within the normal range. In January 2021 the *JAK2V617F* allele burden was 0,018% (22). A panel NGS of 59 genes relevant in myeloid malignancies was performed on the Illumina Novaseq 6000 platform. No pathogenic mutations were detected.

Treatment with ropeg-IFN was fueled by a report, proposing *JAK2V617F* as a new therapeutical target in CVD (21). Accordingly, treatment with ropeg-IFN 125 ug every other week was initiated on April 1 2021. Two weeks later the complaints significantly eased and later completely disappeared (Figure 1). From April 1 to November 1, 2021 the patient received a total of 14 injections every second week. In this period of time the patient was completely free of cardiac symptoms and was well. On November 1, the *JAK2V617F* mutation was no longer detectable and treatment with ropeg-IFN was accordingly paused. By Christmas evening 2021, serious angina reappeared and persisted despite medication as outlined above. A cardiac-CT scan was performed on December 27 which showed slight regression of the coronary artery disease (no in-stent or *de-novo* stenoses) but because of the symptoms treatment with ropeg-IFN was reinstituted (125 ug x 1 sc every second week) in February and the angina attacks disappeared within 2 weeks. On March 4, 2022, a *JAK2V617F* analysis was done, showing an allele burden of 0.012%, which declined to a level of 0.007% on June 16th, when the patient was still feeling well without any cardiac complaints (Figure 1).

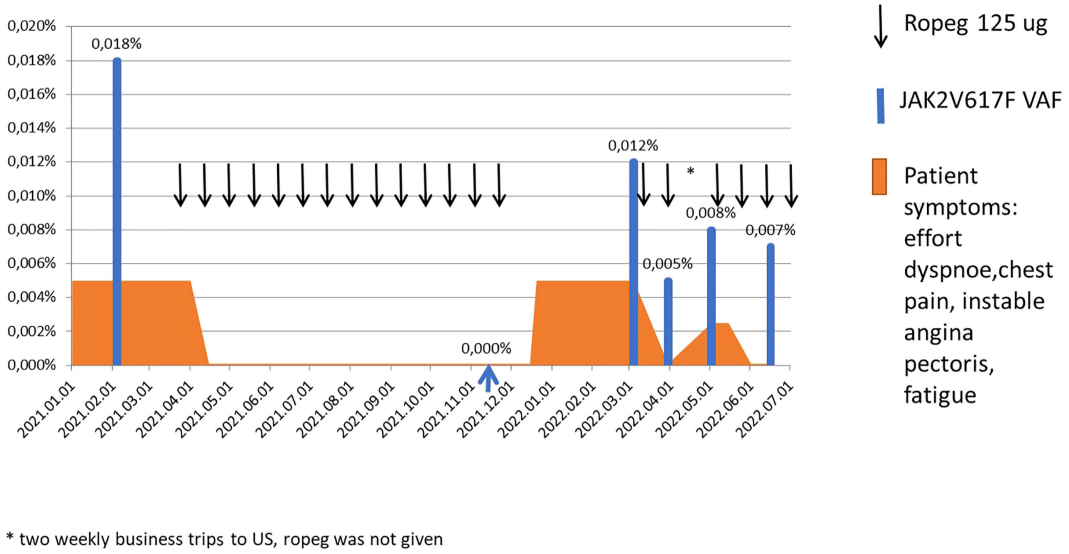

FIGURE 1
Treatment, symptoms and molecular answers.

Discussion

Chronic inflammation is considered an important pathogenetic mechanism for MPN-disease development and disease progression (2, 6, 7, 15). Several thrombo-inflammatory genes have been found to be upregulated, likely contributing to the increased risk of thrombosis (2, 23). In addition, long-term treatment with rIFN normalizes elevated cell counts in concert with induction of a remarkable decrease in the *JAK2V617F* allele burden and accordingly impacting important thrombosis promoting factors in MPNs (2, 8–17). Treatment with rIFN is also able to normo- or significantly downregulate upregulated thrombo-inflammatory genes in patients with MPNs, including PAD4 (23), which strongly induces NETosis and accordingly thrombosis (5). In addition, rIFN downregulates upregulated oxidative stress genes in MPNs (24).

Since CHIP in the general population associates with an increased risk of thrombosis and accordingly CVD it is pertinent to consider if the thrombosis-promoting *JAK2V617F* mutation might be a therapeutic target in the CHIP-stage (13, 21). In this report we have convincingly shown that rIFN rapidly induced resolution of angina pectoris together with a decline in the *JAK2V617F* mutation. Although only a singular observation we believe it to be unique and potentially of utmost importance, hopefully opening the avenue for pilot studies of the impact of rIFN in CHIP-*JAK2V617F* positive individuals with CVD (13, 19, 23). Such studies are even more pertinent, when considering that the *JAK2V617F* mutation associates with thrombosis and ischemic heart disease in the general population (5, 19). These studies should include serial transcriptomic and proteomic studies together with detailed thrombophilia studies in order to elucidate in depth the mechanisms behind the potentially beneficial effects of rIFN upon symptom burden and cardiovascular disease burden in terms of improvement of cardiac function and perhaps also regression of aortic valve calcification, which most recently has been reported to be prominent in patients with MPNs (25) and likely associated with the *JAK2V617F* mutation.

The future treatment of MPNs may imply both stem-cell targeting treatment with rIFN and treatment which targets the chronic inflammatory state, driving clonal expansion in the precursor stage of MPN - CHIP - towards overt MPNs (2, 6, 7, 10, 11, 13, 15). Taking into account the important role of chronic inflammation for clonal expansion both in the CHIP stage and in MPNs, there is an urgent need to investigate, whether stem-cell targeting therapy with rIFN in the CHIP-stage might induce MRD in concert with a reduction in inflammatory biomarkers.

In conclusion, we have for the first time shown rIFN treatment of a *JAK2V617F* positive CHIP patient to induce complete remission of repeated attacks of angina pectoris. We suggest, that the beneficial effects of rIFN might be related to the anti-inflammatory and anti-thrombotic potentials of rIFN, including normalization of elevated blood cell counts, reduction in the *JAK2V617F* allelic burden, dampening of oxidative stress

and decrease in ROS together with downregulation of thromboinflammatory genes, such as the thrombosis-promoting PAD4 and accordingly NETosis activity. Our report calls for clinical studies of the impact of rIFN – either as monotherapy or in combination with potent anti-inflammatory agents upon the cardiovascular disease burden both in the CHIP-stage and in patients with MPNs.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

ME designed research, performed research, analyzed data, wrote the paper. BK and CF performed research. HH,VS, and LK wrote the paper. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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