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Review Article

Aspirin Responsive Erythromelalgia, Cerebral and Coronary Microvascular Thrombotic Manifestations and the 'Early Interferon First Line Intervention strategy' as Curative Treatment Option in Essential Thrombocythemia and Polycythemia Vera

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Abstract

Acetylsalicyl acid (Aspirin^{R} Bayer) cures erythromelalgia and migraine-like microvascular cerebral microvascular ischemic disturbances by irreversible inhibition of platelet cyclooxygenase (COX-1) mediated arteriolar inflammation and platelet thrombi in JAK2^{V617F} mutated thrombocythemia patients with Essential Thrombocythemia (ET) and Polycythemia Vera (PV). Aspirin responsive active platelet prostaglandin metabolism of hypersensitive platelet in thrombocythemia is the mechanism for erythromelalgia and erythromelalgic circulation disturbances to develop caused by acquired or germline gain of function mutations in the TPO, JAK2 and MPL gene and to be labeled as Sticky Platelet Syndrome or Platelet Arterial Thrombophilia. Salicylic acid does not inhibit platelet COX-1 and does not cure aspirin responsive erythromelalgia. Acetylsalicylic acid (Aspirin, synthesized by Hoffmann, Bayer 1897) has been discovered by Michiels between 1975 and 1985 as a wunder drug that cures platelet mediated erythromelalgia and microvascular disturbances. The platelet ADP-receptor inhibitors ticlopedin and clopidrogrel, other platelet affecting agents like dipyridamol, analgetics like sodium salicylate and anticoagulation with coumadin or direct oral anticoagulant (DOAC IIa/Xa-inhibitors) do not inhibit COX-1 activity and are ineffective in the treatment of erythromelalgia and its associated cerebral and coronary ischemic events. Aspirin responsive erythromelalgia and erythromelalgic acrocyanotic and ocular, cerebral and coronary microvascular circulation disturbances in JAK2, MPL or TPO mutated thrombocythemia are alleviated by reduction of platelet count to normal (less than 350x10^{9}/L) with the non-leukemogenic agent pegylated interferon alpha (IFN) to postpone or eliminate the use of the leukemogenic myelosuppressive agents busulphan and hydroxyurea. The 'Early IFN Intervention Strategy' is an effective non-leukemogenic first line curative treatment option in JAK2, CALR and MPL thrombocythemia in ET and PV to improve health care status, quality of life and life expectance by control and reduction of myeloproliferative disease burden.

Keywords: Myeloproliferative Neoplasms; Essential Thrombocythemia; Polycythemia vera; Myelofibrosis; JAK2^{V617F} Mutation; MPL^{515} Mutation; Calreticulin Mutation; Pegylated Interpheron-alpha: Hydroxyurea; Bone Marrow Histology

Introduction

Erythromelalgia is the main pathognomonic and presenting symptom in patients with essential thrombocythemia (ET) and thrombocythemia associated with polycythemia vera (PV). Red congestion and painful swelling and burning frequently affect the extremities of patients with the chronic myeloproliferative neoplasms (MPNS) ET and PV [1]. The condition of "burning painful and red congestion of the foot and hand disorder" was first described in 1878 as erythromelalgia by Silas Weir Mitchell. Erythromelalgia is derived from the Greek words: erythros = red, melos = extremity and algos = pain. In 1938 Smith and Allen substituted the term erythromelalgia for erythermalgia to denote the variability of disease manifestations and to emphasis the importance of heat = therme = warmth as the main important feature of red, burning and painful extremities [2]. The condition, called Mitchell's Disease has been separated by Michiels into aspirin responsive erythromelalgia and microvascular circulatory disturbances in thrombocythemia versus the incurable autosomal dominant congenital primary erythermalgia with bilateral symmetric burning pain in hands and feet in the absence of any underlying detectable disease [1].

Smith and Allen (1938) first described aspirin's effect in promptly relieving burning pain and red congestion in the extremities of erythromelalgia over a period of approximately three days [2]. Michiels established the involvement of platelets' role in aspirin responsive erythromelalgia in thrombocythemia through evidence of platelet consumption, platelet proliferation and increased platelet activation markers obtained through skin biopsy of swollen red areas. Michiels and Van Vliet discovered the specific subset of patients with burning painful red extremities, who experienced clinical relief with aspirin all had elevated platelet counts associated with ET or PV associated with thrombocythemia [3,4]. On behave of the Rotterdam MPD study group Michiels., *et al.* (1985) published their first 10 years experiences that "Erythromelalgia in ET and PV", is caused by platelet mediated arteriolar thrombosis and inflammation that can only be relieved by selective inhibition of platelet cyclooxygenase (COX-1) activity [3]. This platelet-mediated microvascular thrombotic disease (Aspirin responsive Sticky Platelet Syndrome or Platelet Thrombophilia) in the end-arterial circulation of thrombocythemia patients in ET and PV has been studied in great detail by Michiels and Van Vliet between 1975 and 1985 in a series of prospective clinical, histopathological, platelet kinetic and aspirin intervention studies in symptomatic ET and PV patients [5]. Based on skin punch biopsies from erythromelalgia skin areas in ET and PV patients [4,5], Dr Michiels (Hematologist Blood and Coagulation specialist) Dr Ten Kate (Pathologist) and van Dr van Vliet (Coagulation Laboratory Research) of the Erasmus University Medical Center (EUMC 1975-1998) Rotterdam discovered the causal relation that the symptoms of platelet-mediated erythromelalgia, including migraine-like cerebral ischemic attacks and visual disturbances are the result of platelet activation and aggregation *in vivo*, which preferentially takes place in the arteriole of the peripheral cerebral and coronary circulation [3-9].

Aspirin responsive platelet thrombophilia or Sticky platelet Syndrome

The manuscript on "Aspirin responsive Platelet Thrombophilia in ET and PV" by Michiels, Ten Kate and Koudstaal and Van Genderen from the Erasmus University Medical Center (EUMC) Rotterdam in the World Journal of Hematology, 2013 [4] produced a carefully annotated study on the role of aspirin-responsive thrombophilia (Sticky Platelet Syndrome) in ET and PV patients. This state of the art, comprehensive review documents the cellular and vascular impacts of ET and PV and the means to assess and modify the risk of microvascular thrombotic, hemorrhages and major thrombosis. This manuscript painstakingly described the molecular and hematological effects of ET and PV and biologic pathways involved in curing erythromelalgia with reduction of cerebrovascular and cardiovascular ischemic events by aspirin. This EUMC review paper focuses narrowly on the impact of aspirin in reducing erythromelalgia and ocular cerebral and coronary microvascular thrombotic circulation disturbances [3-6]. Aspirin sensitive erythromelalgia is characterized by asymmetric warm, red, congested extremities and painful burning sensations and causally linked to clonal thrombcythemia in various myeloproliferative disorders or neoplasms (MPD/MPN). Paraesthesias of the fingertips toes and forefoot and fingers (acroparaesthesias) like tingling, prickling, numbness, stiffness sensations usually precede the disabling red, swollen burning distress of aspirin responsive erythromelalgia [3-6]. Warmth intensifies the discomfort and cold provides relief. The long-lasting effect of a single dose of aspirin is so specific for erythromelalgia that it can be used as a pathognomonic clue and diagnostic criterion [1-3]. The sustained relief of erythromelalgia and migraine-like ischemic attacks (MIAs) by aspirin is correlated with inhibition of adenoside diphosphate (ADP), epinephrine, collagen and arachidonic acid (AA) induced platelet aggregation as the result of irreversible inhibition of platelet COX1, which can be measured by the malondialdehyde acetate (MDA) in platelet rich plasma (PRP) [1,3-5].

Platelet thrombophilia is a state of circulating hypersensitive platelets labeled as Sticky Platelet Syndrome (SPS) caused by gain of function mutation in the JAK2, MPL or TPO gene [1]. $JAK2^{V617F}$ mutated thrombocythemia in ET and PV is the most frequent cause of SPS, which has been revealed by Michiels and van Vliet as a novel blood platelet abnormality that increases the risk of platelet mediated thrombosis or platelet-rich blood clots in end-arterial blood vessels [1-6]. A core contention of this paper and earlier publications in 1985 and 2006 [3,4] Michiels extensively documented that -complete relief of erythromelalgia and acrocyanotic pain is obtained with the cyclooxygenase inhibitor (COX-1) aspirin (acetylsalicylic acid synthesized by Hofmann Bayer, 1897) - but not with platelet ADP-receptor inhibitors ticlopedin and clopidogrel and also not by sodium salicylate and coumadin [3-6]. COX-1 is an enzyme that is responsible for the formation of platelet prostanoids. The three main groups of prostanoids in JAK2 constitutively activated platelet — prostaglandins endoperoxides and thromboxanes — are each involved in the inflammatory response of red congestion and warmth associated with erythromelalgia [1].

Erythromelalgia, thrombohemorrhagic and Vascular Complications in ET and PV

The vascular complications in PV patients are aspirin sensitive microvascular circulation disturbances typical of thrombocythemia including erythromelalgia, acrocyanotic peripheral ischemia, atypical cerebral ischemic attacks and major arterial and venous thrombosis [1,3-6,9]. Phlebotomy does not prevent the aspirin responsive microvascular ischemic symptoms. The risk of major arterial and venous thrombosis in PV is poorly controlled at increased hematocrits between 0.45 and 0.50 or above [3-6,9]. The risk of microvascular and major thrombosis in PV is best controlled by low dose aspirin on top of phlebotomy keeping the hematocrit around 0.40 and by selection reduction of platelet counts to normal to below 350x10/L in both ET and PV patients [1,3-6,9].

The incidence of thrombotic and hemorrhagic complications in 809 PVSG-defined ET from eleven cohort studies in the literature anno 1997 were analyzed by Dr Griesshammer Germany, Europe

[7]. Thirty-six percent (n=201) were of PVSG-defined ET patients were asymptomatic. Thrombo-hemorrhagic complications were recorded in 59% (n=466) as thrombo-embolic in in 58% (n=466) and hemorrhagic in 17% (n=134), thromboembolic without bleeding in 42% (n=343) and bleedings in 17% (n=134). Van Genderen, Leenknegt and Michiels of the Erasmus University Medical Center (EUMC) Rotterdam produced good evidence that the von Willebrand factor (VWF) is the link between the paradoxical occurrence of bleedings and thrombosis in thrombocythemia of ET and PV patients [8]. The incidence of deep vein thrombosis excluding superficial thrombophlebitis and including portal and splanchnic vein thrombosis in the 809 PVSG defined ET patients was as low as 4% (n=33) [7].

The vascular complications in PV patients are microvascular circulation disturbances typical of thrombocythemia including erythromelalgia, migraine-like atypical ocular and cerebral ischemic attacks (MIAs), and major arterial and venous thrombosis is related to increased hematocrit, erythrocytes and red cell mass and do occur in about two third of PVSG-defined PV patients at time of presentation [4,9,10]. The risk of major thrombosis in poorly controlled PV at hematocrits between 0.45 to 0.50 and above is rather high. The risk of major thrombosis in PV is best controlled by phlebotomy to values around 0.40 [10]. At that hematocrit around 0.40 the fluidity of blood during surgical interventions is most optimal. The microvascular syndrome associated with thrombocythemia in newly diagnosed PV in remission by phlebotomy alone is easily and best controlled by low dose aspirin 40 to 80 mg daily or by selective reduction of platelet count to normal (less than $350x10^9$/L) with low dose non-leukemogenic myeloreductive agents pegylated interferon-alpha2a (Pegasys) and anagrelide or with the potential leukemogenic myelosuppressive agents busulfan or hydroxyurea [4,6,10].

Landolfi, Michiels and Patrono designed between 1994 and 1997 the European Collaboration on Low Dose Aspirin in PV (ECLAP) study [11]. Although Michiels could not contribute patients to the (ECLAP) study "because nearly all PV patients in The Netherlands were on low dose aspirin", Michiels could call on ECLAP data to support his contention that aspirin control of platelet function—coupled with normalizing platelet levels through use of platelet lowering agents "are effective in the prevention of platelet- mediated microvascular circulatory disturbances in thrombocythemia associated with PV [12]. Treatment of PV with low dose aspirin in the ECLAP study as compared to placebo significantly reduced the overall risk of a combined end point of microvascular and major vascular complications, including cardiac death, non-fatal myocardial infarction and stroke and major venous thrombosis from 15.5% to 6.7% during 2.7 years follow-up [12]. Absolute risk reduction was 8.4%. These significant risk reductions in major thrombosis were seen very soon after randomization. Major total and gastro-intestinal bleeding were slightly increased without reaching statistical significance. Michiels., *et al.* extended in the manuscript on "Aspirin responsive platelet thrombophilia in ET and PV" published in the World Journal of Hematology, 2013 [4] their 2000-2019 concept of the ability of the pegylated interferons to effect complete hematological remission and started the discussion on the molecular etiology of platelet mediated thrombosis in ET and PV in light of the new JAK2 mutation findings [13-15].

The 'Early Interferon First Line Intervention Strategy' in the treatment of PV anno 2000 and beyond

In 2000 Lengfelder., *et al*. reviewed the interferon alpha treatment in polycythemia vera patients [17]. On top of low dose aspirin a complete response (CR) in PV implies maintenance of a hematocrit less than 0.45 without the need of phlebotomy and partial response (PR) in PV refers to maintenance of a hematocrit between 0.45 and 0.50 with the need of additional phlebotomy to lower the hematocrit to below 0.45 for major thrombosis prevention. Overall the hematological CR and PR were achieved in 75% of PV cases within 6 months (range 3 to 12 months) in the Lengfelder IFN update of 2000 [17]. About 25% of patients failed a gain of any benefit from IFN because of no response or adverse side effects.

On behave of the 2000 International Polycythemia Study Group (PVSG) Michiels and Silver proposed in 2000 [14] and 2005 [15] a randomized clinical study in stage 1, 2 and 3 PV patients with a clear indication for cytoreduction with biological modifier pegylated IFN-alpha2b (PEG-INTRON) or IFN-alpha2a (Pegasys) to postpone the use of the cytostatic anticancer agent hydroxyurea (Table 1, Source Michiels MPD Book 2005 Doctors Brochure 2004 MPD Workshop Rotterdam) [15]. Treatment algorithm of newly diagnosed ECP defined PV and previously untreated patients with $JAK2^{V617F}$ mutated PV Stage 0 ET (ET with features of PV in blood and bone marrow or prodromal PV), stage 1 erythrocythemic PV and stage 2 early classical PV with no or minor splenomegaly and normal leukocytes less than $10x10^9$/L are candidate for low dose aspirin and phlebotomy (Table 1). Symptomatic stage 0 and stage 2 symptomatic PV patients increased leukocytes above $10x10^9$/L complaining of fatigue, itching or uncontrolled platelet counts and bleeding are candidates for low dose pegylated INF-alpha2a (Pegasys) for correction of platelet and leukocytes to normal to postpone or eliminate the use of hydroxyurea or busulfan as long as possible (Table 1, Source Doctors Brochure and MPD Workshop Rotterdam 2004, Michiels Kvasnicka and Thiele 2005) [14,15]. According to Michiels and Silver a randomized clinical trial directly comparing rIFN-alpha2a (Pegasys) versus hydroxyurea in newly diagnosed PV patient stage 1 2 and 3 was not ethical at that time anno 2004 [14-16]. Kiladjian opted and designed in 2003 for IFN-alpha2a (Pegasys) the Paris Nord PV study in consecutive newly diagnosed PV patients irrespective of age as the first curative option in early stage PV and if IFN non-responsive to or intolerant to opt for hydroxyurea [13,14].

Table 1A: The Six stages of polycythemia vera according to Wasserman 1995 and Michiels 2004.

Table 1B: Proposed study design for stratification of previously untreated PV patients.

IFN first in ECP stage 1 and 2 and IFN vs hydroxyurea (HU) in ECP stage 3 PV (Table 2) at age 50-65 and Hydroxyurea first line at age above 65 years. Designed by Michiels 2004.

The predicted therapeutic benefits of first line curative treatment of PV with pegylated IFN-alpha2a (Pegasys) include hematological remission with significant improvement or correction of platelet and leukocyte counts to normal, correction of iron deficiency state, resolution of PV disease-associated symptoms itching in particular, resolution of minor or moderate splenomegaly to normal sizes, relief from hemorrhagic events by reduction of platelet count from above to below $1000x10^9$/L and relief from microvascular disturbances by correction of platelets to below $300x10^9$/L [13,15,16]. The predicted required IFN-alpha2a (Pegasys) dose in early symptomatic stage 3 PV is low with much less flu-like side effects and does not interfere with the working and recreation ability on the long term [15]. Side effects remain a significant problem in over 30% of PV patients. About 20% of PV patients discontinue IFN-aplpha2a (Pegasys) therapy due to intolerance. The rational to use IFN in newly diagnosed PV patients anno 2005 included: abatement of constitutional symptoms, maintenance of hematocrits to below 0.45, platelet and leukocyte counts to normal or nearly normal, avoidance of phlebotomy, iron deficiency, and macrocytosis, mucocutaneous lesions of hydroxyurea, and very importantly lack of leukemogenicity of hydroxyurea [15-17].

The rational for the 'Early Interferon Intervention' curative option of rIFN-aplpha2b (PEG-INTRON) or IFN-alpha2a (Pegasys) has been established during the first EWG.MPD Workshop in Rotterdam 1998 and appeared to be related to the observed much more favorable efficacy safety and toxicity profile of pegylated INF as compared to hydroxyurea (Table 2) [14-16]. An open label randomized treatment program as proposed by Michiels between 2000 and 2005 would establish the efficacy and safety of pegylated IFN-alpha2a (Pegasys) in early stage PV plus adjuvant hydroxyurea in IFN not responsive cases as compared to standard phlebotomy/low dose aspirin plus anagrelide if needed for the control of platelets in previously PV patients has never been performed and is difficult to carry out in routine daily practice [14]. Since 2000 Michiels predicted that pegylated IFN-alpha2a (Pegasys) is a promising alternative to postpone the use of hydroxyurea, but IFN is expensive and its side effects in about 30% are unbearable (Table 2) [14,15]. In the Netherlands Michiels followed the Interferon-alpha (IFN-α) first line treatment option in PV because it may reduce and prevent progressive MPN disease burden if used early in the early prefibrotic symptomatic stage of prodromal PV and overt PV disease [4,13-15]. Clinicians are reluctant to postpone the use of hydroxyurea in early stage PV as long as it is a conservative approach using phlebotomy aiming at a hematocrit around 0.40, on top of low dose aspirin for the control platelet function, and if indicated, anagrelide for the control of platelet and leukocyte levels (Table 2) [13-16].

IFN-alpha (IFN-α) has broad effects on the hematopoietic system. IFN inhibits erythroid progenitors and erythroid burst-forming units (BFUEs) *in vitro* and produces morphologic and biochemical changes in megakaryocytes, including a decrease in megakaryocyte density and size, presumably affecting megakaryocyte maturation and platelet release. In PV, biochemical abnormalities characterized by impaired conversion of exogenous arachidonic acid by platelet thromboxane B2 and correction of that deficiency has been reported. The use of IFN antagonizes platelet-derived growth factor (PDGF). When compared with nonspecific myelosuppressive drugs hydroxyurea and busulfan [1,13,14], recombinant IFN is a biological modifier of hematopoiesis and represents a non-leukemogenic modality that may fundamentally alter the course of the PV disease, and thus offers a physiologic basis for its use in PV [14,16].

The 'Early Interferon First Line Intervention Study' in PV, USA 1994-2005

Silver reported in 2004 during the Second MPD Workshop Rotterdam the use of rIFN-alpha2b (PEG-INTRON) in 55 patients with PV, treated at his institution over the past 15 years. The results of rIFN-2b treatment in 55 PVSG defined PV patients, who reached complete hematological responses defined by freedom of phlebotomy, hematocrit below 0.45 and platelet count below $600x10^9$/L and reduction of enlarged spleen sizes are shown in Figures 2 and

PV: CLMP stage	0	1	2	3	4	5	6
Clinical Diagnosis	**Prodromal PV**	**Erythro-cythemia**	**Early PV**	**Classical PV**	**Masked PV**	**Post-PPV MF Inapparent PV**	**Post-PV MF Spent phase**
LAP-score, CD11B	↑	↑	↑	↑	↑/↑↑	↑	Variable
EEC	+	+	+	+	+	+	+
Red Cell Mass	N	N	↑	↑/↑↑	↑/↑↑	↓ or ↑	Variable
Erythrocytes $x10^{12}$/l	<5.8	>5.8	>5.8	>5.8	N	N	↓
Leukocytes $x10^{9}$/l	<12	<12	<or >12	< or->15	>15	N or ↑	>20
Platelets $x10^{9}$/l	>400	400	< or >400	>400	+1000	N or ↑	Variable
Bone marrow histology	EM	EM	EM	EMG	EMG	MG-MF	MF
BM cellularity (%)	50-80	50-80	60-80	80-100	80-100	60-100	↓
Grading RF	RF 0-1	RF 0-1	RF 0-1	RF 0/1,	RCF2/3	RCF 2/3	RCF 3/ 4
Grading MF[57]	MF 0	MF 0	MF 0	MF 0	MF 1 2	MF 1 2	MF 2/3
Spleen size							
On echogram	<12-15	<13	12-15	12-16	18->20	16 >20	>20 cm
Below MCL	0-3	NP	0-3	4-6	>6	>6	>8 cm
$JAK2^{V617F}$ mutation load	Low	Low	Moderate <50%	Mod/High	High >50%	High >50%	High >50%
Granulocytes %	+(++)	+(++)	+	+ / ++	++	++	++
CLMP Risk stratification	Low	Low	Low	Intermediate	High	Post-PV MF	Post-PV MF
→Therapeutic implications	Aspirin (Asp)	Asp/Phleb IFN?	Asp Phleb IFN	Asp/Phleb IFN	IFN, if non responsive HU-JAK2 inh	JAK2 inhibitor Ruxolitinb	Allogeneic BMT

Table 2: 2014 ECMP and 2018 CLMP criteria for staging of $JAK2^{V617F}$ mutated myeloproliferative neoplasms (MPNs) prodromal PV, erythrocythemic PV, early PV, classical PV, masked PV, inapparent PV and post-PV myelofibrosis (MF) and grading of myelofibrosis: Therapeutic implications. Designed by Michiels [25].

*↑: Increased; ↓: Decreased; N: Normal, +: Present or Heterozygous; ++: Homozygous; Derived from RCT: Randomized Clinical Trial.

3 (Personal communication 2004 MPD Workshop Rotterdam). The platelet count in the 1st, 2nd, 3rd and 4th quartile ranged from 138-394, from 417-536, from 570-703 and from 732-1340 respectively. There were no thrombo-hemorrhagic complications during follow-up of a few months to 2 years in 18 PV patient and during follow-up from 2 to more than 10 years in 37 PV patients. The toxicity profile in these 55 PV patients were initial fluelike symptoms in all, fatigue in all, liver function abnormalities in 4, peripheral neuritis in 1, hypothyroid in 1 and severe asthenia in 1. Reasons for discontinuation of rIFN-alpha2b (PEG-INTRON) therapy were toxicity in 4, sustained remission in 2, no health insurrance coverage in 1, and malignancy in 1. Three PV patients developed myelofibrosis after 87, 96 and 129 months, of whom two required additional hydroxyurea and 1 received prednison.

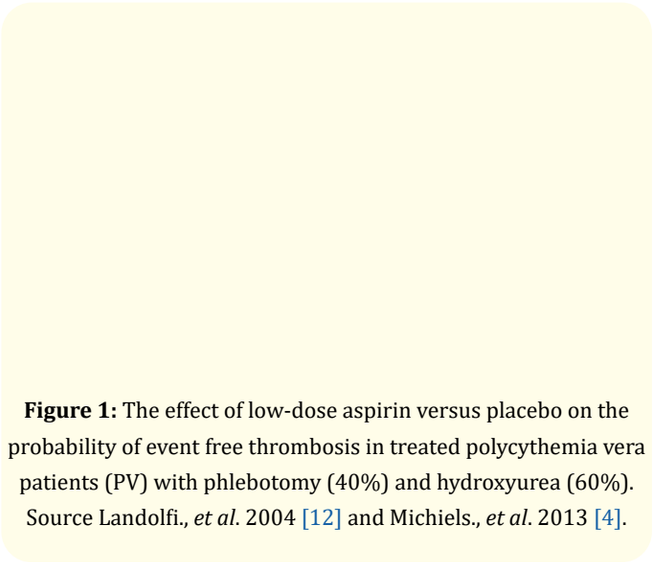

Figure 1: The effect of low-dose aspirin versus placebo on the probability of event free thrombosis in treated polycythemia vera patients (PV) with phlebotomy (40%) and hydroxyurea (60%). Source Landolfi., *et al*. 2004 [12] and Michiels., *et al*. 2013 [4].

The 'Early Hydroxyurea First and IFN Second Line Intervention Strategy' in PV, USA 2000-2019

Since 2000 and in their yearly updated educational papers in Am J Hematol and many other medical journal– Tefferi and Barbui mentioned IFN-alpha as a difficult agent as compared to hydroxyurea in terms of patient counseling in routine daily practice and therefore preferred the use hydrxoxyurea in ET and PV for correction and control of increased increased platelets, leukocytes and/or erythrocytes in ET and PV [16,18-30]. The WHO recommendations on the use of hydroxyurea in PV and ET include an age and thrombotic risk stratification system, allowing patients with low thrombotic risk at age below 60 years, no prior thrombosis, platelets less than$1500x10^{9}$/L – to be followed without any cytoreductive

Figure 2: 2004 Update of rIFN-2b treatment in 50 PV patients by Dr Silver.
Courtesy of Dr Silver MPD Workshop Rotterdam, 2004.

Figure 3: The effect of rINF-2b on spleen size reduction in 12 PV patients in the study of Dr Silver. Courtesy of Dr Silver, MPD Workshop Rotterdam 2004.

hydroxyura therapy but only being treated with aspirin in ET/PV on top of phlebotomy in PV aiming at a hematocrit below 0.45 [18]. Low thrombotic risk ET and PV patients reaching 60 years of age will become candidates for life long hydroxyurea. (over)treatment therapy. ET/PV patients when they had a previous or succumbed a major thrombosis or hemorrhage irrespective of MPN disease burden were treated with upfront hydroxyurea [18]. In most "non-IFN-access" centers in most countries of the World outside Europe-about 85% of these ET and PV patients are actually treated with upfront hydroxyurea according to WHO guidelines. Hydroxyurea is a potentially leukemogenic agent when being used for long periods with increased risk of acute myeloid leukemia (AML) /myelodysplasia (MDS) of 10% after about 10 years, 15% after about 15 years and 20% after about 20 years[16].

The Myeloproliferative Disorders (MPD) Research Consortium (MPD-RC) recently published the investigator-initiated, international,multicenter, phase 2 trial evaluating the ability of pegylated IFN-aplpa-2a (Pegasys) therapy to induce complete and partial hematologic responses (CR/PR) in high-risk ET/PV patients, who were either intolerant or refractory or intolerant to hydroxyurea. Intolerant to hydroxyurea means discontinuation of hydroxyurea duet o side effects. Refractory to hydroxyurea in classical and hypercellular PV is much less frequent and usually points to advanced stages of PV and ET or masked PV. The MPD Research Consortium study included 65 patients with ET and 50 patients with PV [29]. The overall response rate (complete response/ partial response: CR/PR) at 12 months IFN-2a (Pegasys) treatment was 69.2% (43.1%/26.2%) in WHO defined ET, and 60% (22%/ 38%) in WHO defined PV patients intolerant or resistant to hydroxyurea. Complete response (CR) rates were significantly higher in CALR mutated ET patients without features of PV (56.5% versus 28.0%, p= 0.01) as compared to subjects lacking a CALR mutation and carrying the $JAK2^{V617F}$ mutation in PV and ET patients. The median absolute reduction in $JAK2^{V617F}$ variant allele fraction (VAF) was -60% (range -84%-47%) in patients achieving a complete remission versus +4% (range -18%-56%) in patients with partial remission/non-response (PR/NR). IFN-2a (Pegasys) therapy was associated with a significant rate of adverse events, most were manageable, and IFN-2a (Pegasys) discontinuation due to adverse event occurred only in 13.9% of ET and PV patient subjected to IFN-2a (Pegasys) treatment for 12 months. The MPD Research Consortium concluded in september 2019 that pegylated IFN-2a (Pegasys) is an effective therapy for patients with ET/PV who were previously refractory and/or intolerant to hydroxyurea [29]. Applying The Hydroxyurea First Line Intervention Strategy in the MPD Consortium Research Consortium study, only a small proportion of newly diagnosed ET and PV patients will receive upfront IFN, whereas the about two third of PV and ET in The IFN First Line Treatment Strategy in the Kiladjian study are candidate for upfront IFN with complete and good hematological responses (CR/PR) during long-erm or even life-long follow up not needing hydroxyurea treatment anymore (Table 2, Figure 4).

Figure 4: Overall $JAK2^{V617F}$ molecular responses to IFN-alpha2a Pegasys treatemt in 37 PV cases treated between 2004 and 2011. Courtesy of Dr Kiladjian American Society of Hematology: ASH 2012.

The 'Early Interferon First Line Intervention Strategy' in PV to postpone or eliminate the use of hydroxyurea 1999-2019

The European Working Group on Myeloproliferative Disorders (EWG.MPD founded and chaired by Dr Michiels 1994-2006) extended and modified the PVSG diagnostic criteria for PV, ET and

PMGM by including bone marrow histopathology as a pathognomonic clue to each of the MPDs caused by the driver mutations JAK2, CALR and MPL [16,24-28]. From the results of prospective randomized trials in previously untreated PV patients it became evident that new clinical trials in previous untreated PV patients anno 1999 [13,14] should focus on IFN-alpha, a non-leukemogenic approach versus a conservative approach in PV as long as a conservative approach using phlebotomy aiming at a hematocrit around 0.40, plus low dose aspirin for the control of platelet hyperfunction or anagrelide for the control of platelet number [14]. The Polycythemia Nord-1 (PVN-1) study from Paris was designed by Jean Jacques Kiladjian as the spinoff of the First MPD Workshop 1998 in Rotterdam and followed the 2000 EWG.MPD recommendations [13]. The PVN-1 is a multicenter open phase 2 study of pegylated IFN-alpha2a (Pegasys) of 40 PV patients from 12 centers, in which 40 PV patients were included from September 2004 to September 2005 of which 3 of them were not evaluable for long-term intention to treat (ITT). Thirty-seven (37) PV patients were treated with IFN-2a (Pegasys) up to 2011 with a median follow up of 75 months (Figure 4). No vascular events while on low dose aspirin were recorded. Twenty-six (26) PV patients (70% of 37) had stopped IFN-2A (Pegasys) after a median of 4 months. Reasons for discontinuation of IFN-2a (Pegasys) in 26 PV patients were: Complete hematological response (CHR) in 14 (38% of 37); toxicity in 10 (27% of 37); ad 2 (5% of 37) for other reasons. Eleven PV (30% of 37) were still on maintained IFN-2a (Pegasys) for a median follow-up of 70 months. The 14 PV patients discontinued IFN-2a (Pegasys) after a median folllow-up 31 months (range 6 to 66 months indicating a spectrum of rapid and slow responders to IFN-a (Pegasys). Ten PV patients remained in CHR (27% of 37) and 4 PV had a relapse. Eight (8) PV patients (22%) achieved a complete molecular response (CMR), the cummulative CMR was reached at 2 to 4 years of IFN-2a Pegasys treatment. The overall $JAK2^{V617F}$ responses in 37 PV treated with IFN-2a Pegasys between 2004 and 2011 is shown at time point 2008 in figure 4 and at time point 2011 in figure 5 (Source Kiladjian Personal communications ASH 2012) [19,20]. "The rational for using IFN-α as the first-line curative treatment option in newly diagnosed PV-patients include its effectiveness to abate constitutional symptoms and to induce a complete remission, thereby avoiding phlebotomy, iron deficiency and macrocytosis associated with hydroxyurea [4,13-21]. A key observation is that IFN-2a (Pegasys) indeed did normalize bone marrow histology in those cases who reached a complete molecular response as documented in bone marrow biopsy slides in the study of Larsen., et al. (Figure 5) [21] and in the study of Kiladjian (Figure 6) [20], The 'Early IFN Intervention Concept' of Michiels, Siver and Hasselbalch proposed in 1999 [13] was confirmed by the dramatic favorable effect of IFN-alpha Pegasys inducing major molecular responses in newly diagnosed and previously untreated PVSG-defined PV patients in the study Kiladjian's groundbreaking work studies showing that maintained IFN-alpha induced progressive reduction of the $JAK2^{V617F}$ mutation allele burden from above 50% to less than 10% indicating homozygosity during 3 to 5 years follow-up and subsequent elimination of hydroxyurea during lifelong follow-up in complete and good responders to IFN2a (Table 2, Figure 4) [19,20].

Figure 5: Reversal of a typical PV hypercellular bone marrow due to increased eythrovytic and mrgakarocytic proliferation into a normocellular bone and normal megakaryocytes in PV case after 8 years of IFN-2a (Pegasys) treatment in the study of Larssen., *et al.* [21].

Figure 6: The effect of IFN2a (Pegasys) in three cases of PV in complete hematological response in the study of Kiladjian., *et al.* 2012 [20].

Very good evidence in support of the 'Early Interferon Intervention Concept' in ET and PV patients has recently been produced by Dr Hans Hasselbalch Department of Hematology, Zealand Hospital, Sygehusvej 10, 4000 Roskilde, Danmark [21,22]. In the 2018 Leukemia manuscript of Czech., *et al.* [23], Hasselbalch and Koschmieder performed a retrospective study of 38 MPN patients (Table 3) from medical records treated with IFN-2a (Pegasys) at the Roskilde Hospital Danmark and compared the efficacy of pegylated IFN-alpha2a (Pegasys) treatment in ET and PV patients with $JAK2^{V617F}$ and CALR mutated myeloproliferative Neoplasms (MPN). Retrospective analysis of IFN-2a (Pegasys treated MPN patients revealed that $JAK2^{V617F}$ mutated ET (N=18) and CALR mutated ET N=20) show a similar complete and good clinical hematological response whereas $JAK2^{V617F}$ mutated ET or PV patients are more likely to achieve a partial molecular response (PMR) or no molecular response (NR) in 61% and 22% respectively as compared to CALR mutated ET patients reaching PMR and NR in 20% and 70% respectively (Figure 7). The overall hematological response (HR) was

94% for JAK2^{V617F} and 89% for CALR mutated MPN patients. The complete hematological response (CR) rates (correction of platelet and leukocyte count, Table 3) rates were 88% for JAK2^{V617F} and 84% for CALE mutated MPNs. Three of 36 patients with elevated platelet or leukocyte counts.

Table 3: Clinical and laboratory characteristics of 18 JAK2^{V617F} and 20 CALR mutated patients with 2016 WHO defined essential thrombocythemia (ET), Prefibrotic Primary myelofibrosis (PrePMF) or overt PMF treated with pegylated INF-2a (Pegasys) at the Roskilde Hospital in Danmark. Source Czech et al Leukemia 2018, Springer Nature.

Figure 7: a. Reduction in JAK2^{V617F} in PV and CALR mutation load in ET patients before and after IFN-alpha treatment in the study of Czech., *et al.* [23]. c. Partial Molecular Responses (PMR), major Molecular Responses (mMR) and No Response (NR) in JAK2^{V617F} mutated PV and in CALR mutated ET in the study of Czech., *et al.* [23]. b. The degree of mutation allele burden by IFN-alpha treatment in JAK2^{V617F} mutated PV and in CALR mutated ET patients in the study of Czech., *et al.* (23]

Discussion and Conclusion

About 25 years ago recombinant interferon-alpha was used successfully for the first time in the treatment of essential thrombocythemia and a few years later in the treatment of polycythemia vera and hyperproliferative WHO defined prefibrotic myelofibrosis (prePMF). A large number of European studies and reviews – documenting that interferon is not " an experimental drug" in selected early to intermediate PV patients [22]. Interferon is an old horse in the circus which – for authorative reasons – has been neglected and dismissed in the USA, UK, Belgium and Italy. The MPNs experts– Dr Silver in the 1990s [13,14] and Dr Kiladjian from Paris [19,20] as well as MPN colleagues in Danmark, Sweden, Norway Central European countries between 2000 and 2019 have collected a huge amount of clinical, experimental and basic research experiences in the use of IFNs in MPNs with up to at least 20 years follow-up in at least hundreds of patients treated with IFN during these years. As low dose aspirin in ET and low dose aspirin on top of phlebotomy in PV did not prevent MPN disease progression into hypercellular ET and PV, there is very good clinical and experimental evidence that the 'Early Interferon Intervention Strategy' (Table 2) will be validated in ongoing studies and soon will become the first line treatement option in ET, prodromal PV and PV as an effective non-leukemogenic first line treatment option in ET and PV patients for several reasons. We predict that the MPN patients and Doctors communities will have access to IFN-alpha2a and IFN-alpha2b in 2020 as the first line treatment option for newly diagnosed symptomatic ET, prodromal PV and classical PV patients with the likely outcome that their counts steadily decline within weeks or months and in the JAK^{2V617F} positive patients with a decline in the JAK2-allele burden as well (Table 2). [24-26] This is fortunate, since the JAK2^{V617F} allele is a platelet-mediated thrombosis promoter per se and – according to some epidemiological studies – a tumor promoter as well (Table 2). Furthermore, the JAK2^{V617F} allele burden induces increasing genomic instability predisposing to additional mutations, subclone formation, resistance to treatment and ultimately leukemic and myelofibrotic transformation. The 'Early Intervention First Line IFN Concept' to combat MPN disease as early as possible and prohibit cancer progression is currently increasingly prevailing in Nordic and Central European countries to postpone and abandon hydroxyurea and even believe based on supporting data that hydroxyurea may not only elicit AML /MDS and solid tumors as well. This makes sense when realising that hydroxyurea impairs DNA repair mechanisms and tumor suppressor function (p53) as well [22]. Anno 2019 it is very exhausting and distressing for MPN-patients that the "Hematological Scientific Community" are not yet but hopefully will become sensitive to uniformly take notice of the vast amount of convincing data showing that IFN is a highly potent agent (Table 2), which has already changed the lives of hundreds to thousends of MPN-patients worldwide – even inducing minimal residual disease. Hasselbalch is prepared to introduce studies in Denmark combination IFN/suppressive therapy and offering IFN-intolerant patients combination therapy with Ruxolitinib (Jakafi).

Low dose IFN-alpha normalizes peripheral leukocyte and platelet counts within a few months in the large majority of patients with classical ET, PV and hyperproliferative prefibrotic PV [24-28]. Low dose IFN-alpha decreases the need for phlebotomies in the large majority of PV patients within the first 6-12 months but still some patients need phlebotomies in the second and third year. Low dose IFN-alpha is well tolerated by the large majority of patients. One third of newly diagnosed JAK2 mutated prodromal and classical PV patients are complete hematological responders with a major molecular response (MMR), another one third are complete hematological responders with a partial molecular response (PMR) to JAK2 allele burden below 5%. One third of PV patients at time of presentation do not tolerate IFN-alpha very well. IFN non-responsive PV patients are candidates for secondline treatment option with hydroxyurea to correct blood cell counts. Ruxolitinib is a good alternative or the best option in hypercellular advanced PV to reduce increased MPN disease burden which is associated with relief of constitutional symptoms and significant reduction of spleen size (Table 2) [4,18]. Hasselbalch and his team of clinical and basic research investigators in Danmark are the proponents in favor of the effectiveness of low dose IFN-alpha in newly diagnosed $JAK2^{V617F}$ mutated ET and PV patients and CALR ET patients and promoted the 'Early Interferon Intervention Strategy"for several reasons. First,low dose IFN is able to induce regression of MPN disease burden including spleen size, itching and myelofibrosis in a subset of patients with hyperproliferative myelofibrosis (MF). Second, low dose IFN induces deep molecular remissions as assessed by the $JAK2^{V617F}$ allele burden and normalisation of the bone marrow histology [4] – being sustained even after discontinuation of IFN for up to 5 years aswill be published by the Danish MPN-group (manuscripts in preparation). Third, low dose IFN is the only agent having the potential to induce "minimal residual disease " and "operational cure " in MPNs.\ during long-term and even life long follow-up (Figures 2 and 3) [16-19]. Fourth, low dose IFN reduces the hard end points – microvascular disturbamces, major thrombosis and hemorrhages – because of correction of platelet and leukocyte counts to normal levels as compared to historical controls. Finally, low dose IFN-alpha is Pegasys 45 ug x 1 subcutaneously (sc) per week equivalent but superior to PegIntron 30 ug x 1 sc/week.

The statement of Hasselbalch [22] that we have to rethink the academic approach that searching for the highest level of evidence – the results from a randomised trial comparing hydroxyurea to interferon in high risk patients (The MPN-Consortium Study USA [29]) is not appropriate and ethical anymore. The "Wait and Watch" approach untill there is a clear indication for hydroxyurea in PV patients with progressive MPN disease is fishing behind the net and may became a disaster for the affected PV patient since we know now for sure that a substantial proportion of our MPN patients during the progressive course of the disease will suffer major thrombotic and/or hemorrhagic events. Without the use of IFN as a first line treatment option, we know, that the MPN diseases – as all other cancers – will steadily evolve along the inevitable path from an early myeloproliferative stage (ET/PV) to the advanced ET and PV stage – myelofibrosis – when there is no way back for most patients. Instead, we should all put our efforts together to let dispear the barriers between the two worlds – to fuse our concept insight that the recent pegylated IFN-2a study in Blood by Yacoud., *et al* [29] confirmed and validated that currently is the first line treatment option in symptomatic $JAK2^{V617F}$ mutated ET, prodromal PV, classicical PV and CALR mutated thrombocythemia patients at time of presentation. If non responsive to IFN the cornerstone of today's treatment of ET and PV with progessed MPN disease like hypercellular PV are candidates for hydroxyurea and or the JAK2-inhibitor Ruxolitinib or other targeting agents displaying a synergism with IFN at time points before intermediate and advanced myelofibrotic transformations do occur [26]. In a post-hoc study of 83 ET/PV patients, Masarova & Verstovsek showed in 2017 that IFN-alpha2a (Pegasys) induced durable hematological remissions in 80% of 83 ET/PV patients and molecular responses in 35 of 55 (63%) of ET/PV patients during long-term periods lasting from 5.7 to 12 years [30]. Finally, Michiels and Hasselbalch did see several PV patients on life-long IFN from the USA and Europe, who consulted and visited us for our advice on IFN telling us: This interferon is really working for me! You saved my life.

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