Interferons in MPNs

Perspectives on The Early Interferon Concept

Combination Therapy with Ruxolitinib and Interferon

Hans Hasselbalch
Department of Hematology
Roskilde Hospital
University of Copenhagen
Denmark

Bologna
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Interferon Alpha2 in MPNs

- ✓ **History of Interferons**
- ✓ Biology and Mechanisms of Action
- ✓ The Novel Concept of Chronic Inflammation in MPNs
- ✓ Early Intervention Concept
- ✓ Minimal Residual Disease
- ✓ Interferon Resistance and Intolerability
- ✓ Perspectives – Combination Therapy with Ruxolitinib

Honorary President
Sante Tura

- Interferon-Alpha2 in Chronic Myelogenous Leukemia
- Interferon-Alpha2 in Essential Thrombocythemia and Polycythemia Vera
- Interferon-Alpha2 in Hairy Cell Leukemia
- Interferon-Alpha2 in Malignant Lymphoma
- Interferon-Alpha2 in Chronic Lymphocytic Leukemia
- Interferon-Alpha2 in Mycosis Fungoides
- Interferon-Alpha2 in Cutaneous T-Cell Lymphoma
- Interferon-Alpha2 in BCR-ABL Positive Acute Lymphoblastic Leukemia
- Interferon-Alpha2 in Multiple Myeloma
- Interferon-Alpha2 in Immune Thrombocytopenic Purpura

66 Papers from 1987-2004

Gugliotta L, Macchi S, Catani L, Chetti L, Mattioli Belmonte M, Guarini A, Criscuolo D,Tura S. Recombinant alpha-2a interferon (alpha-IFN) in the treatment of essential thrombocythaemia.. Haematologica. 1987 May-Jun;72(3):277-9

”New Drugs in Hematology”

INTERFERON

- ✓ 1957 : IFN is the first cytokine discovered (Isaacs & Lindemann)
- ✓ 1978 : purification,analyses and characterization
- ✓ 1980 : Cloning of recombinant human IFN-alpha and beta
- ✓ 1983 : First report of efficacy in CML
- ✓ 1985 : First report of efficacy in ET — Linkesch W & Gisslinger H
- ✓ 1986 : FDA approval for treatment of HCL
- ✓ 1987 : First report of efficacy in MF — Parmeggianni L et al
- ✓ 1988 : First report of efficacy in PV — Silver RT

- ✓ 2016 : No approval for treatment of MPNs

Burning Questions

Why interferon ?

When Interferon ?

Why Interferon-Alfa ?

30 Years of Clinical Experience
Single Arm Studies
> 1000 Patients

Safe

Efficaceous

When Interferon-Alfa ?

From The Time of Diagnosis

Tumor Burden at The Minimum

Most Efficaceous

Interferons
in Ph-Negative MPNs

- ✓ Interferon alpha-2b (Introna/PegIntron)
- ✓ Interferon alpha-2a (Pegasys)
- ✓ AOP2014, a Novel Peg-Proline-Interferon Alpha-2b

The renaissance of interferon therapy for the treatment of myeloid malignancies

Jean-Jacques Kiladjian,[1-3] Ruben A. Mesa,[4] and Ronald Hoffman[5]

Centre d'Investigations Cliniques, Hôpital Saint-Louis, Assistance Publique–Hôpitaux de Paris, Paris, France; [2]Université Paris Diderot–Paris 7, Paris, France; [3]Inserm, CIC 9504, Paris, France; [4]Mayo Clinic, Scottsdale, AZ; and [5]Tisch Cancer Institute Mount Sinai School of Medicine, New York, NY

Table 3. Clinical trials of interferon in essential thrombocythemia

First author, year	No. of patients	Response rate, %
Giles, 1988	18	100%
Bellucci, 1988	12	NA
Gugliotta, 1989	10	100
Lazzarino, 1989	26	86
Giralt, 1991	13	69
Gisslinger, 1991	20	85
Sacchi, 1991	35	85
Turri, 1991	10	70
Seewann, 1991	19	80
Kasparu, 1992	14	86
Rametta, 1994	25	92
Berte, 1996	12	83
Sacchi, 1998	11	100
Radin, 2003	17	88
Alvarado, 2003	11	100
Saba, 2005	20	75
Langer, 2005	36	75
Samuelsson, 2006	21	70
Jabbour, 2007	13	70
Quintas-Cardama, 2009	39	81

Table 2. Clinical trials of interferon in polycythemia vera

First author, year	No. of patients	Reduction of PHL, n (%)	Freedom from PHL, n (%)
Cacciola, 1991	11	9 (82)	5 (45)
Cimino, 1993	13	10 (77)	4 (31)
Finelli, 1993	13	11 (85)	11 (85)
Turri, 1991	11	7 (64)	4 (36)
Papineschi, 1994	11	9 (82)	8 (73)
Sacchi, 1994	22	21 (95)	21 (95)
Muller, 1995	15	7 (47)	NA
Taylor, 1996	17	14 (82)	9 (53)
Foa, 1998	38	19 (50)	11 (29)
Gilbert, 1998	31	NA	NA
Stasi, 1998	18	17 (94)	11 (61)
Heis, 1999	32	28 (87)	2 (6)
Radin, 2003	12	5 (42)	1 (8)
Silver, 2006	55	55 (100)	53 (96)
Samuelsson, 2006	21	7/9 (78)	4/9 (44)
Kiladjian, 2008	37	37 (100)	36 (97)
Quintas-Cardama, 2009	40	32 (80)	28 (70)

. **Kiladjian JJ, Mesa RA, Hoffman R. Blood. 2011 ; 5;117(18):4706-15**

The renaissance of interferon therapy for the treatment of myeloid malignancies

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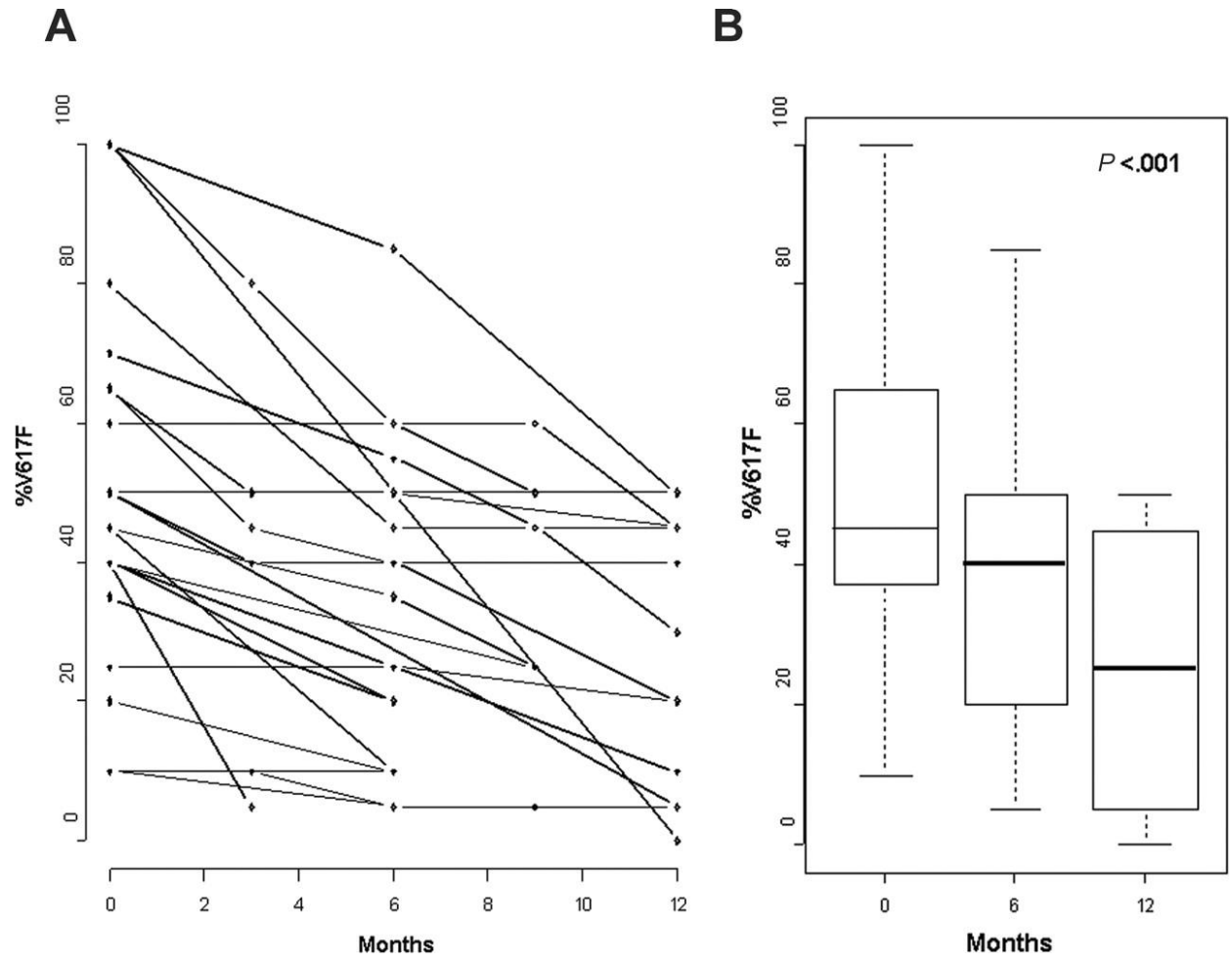
Table 4. Clinical trials of interferon in myelofibrosis

First author, year	No. of patients	Response rate, %	Spleen size reduction, % of patients
Hasselbalch, 1988	10	0	10
Barosi, 1989	10	25	0
Gilbert, 1998	22	NA	58
Tefferi, 2001	11	0	18
Radin, 2003	31	3	33
Jabbour, 2007	11	9	NA
Ianotto, 2009	18	44	11
Silver, 2009	13	38	38

. **Kiladjian JJ, Mesa RA, Hoffman R. Blood. 2011 ; 5;117(18):4706-15**

Interferon-Alpha2 Significantly Reduces The JAK2V617F-Allele Burden



Kiladjian, J.-J. et al. Blood 2006;108:2037-2040

Interferon Alfa Therapy in CALR-Mutated Essential Thrombocythemia

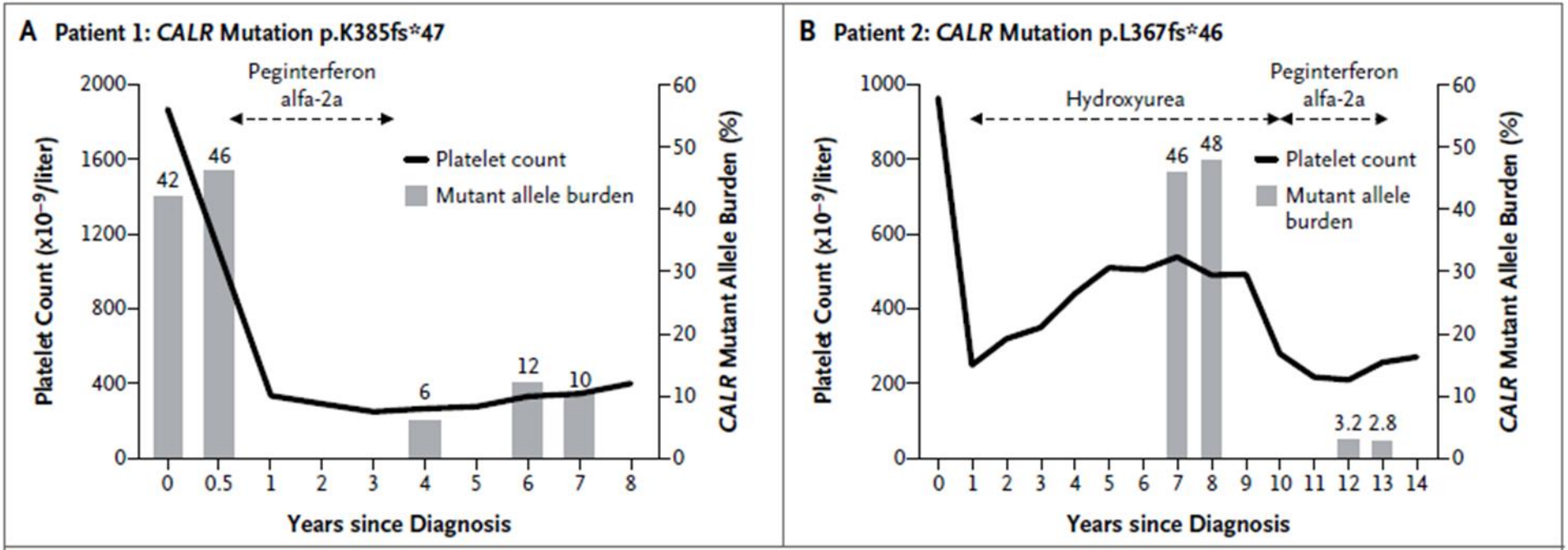


Figure 1. Evolution of Platelet Count and *CALR* Mutation Burden during Treatment in Two Patients with Essential Thrombocythemia.

The treatment periods with hydroxyurea or peginterferon alfa-2a are indicated by arrows. *CALR* mutations were identified with the use of direct Sanger sequencing, and the *CALR* mutant allele burden was calculated with the use of DNA fragment analysis and area-under-the-peak measurement: % of *CALR* mutant allele burden = (mutated *CALR* ÷ [nonmutated *CALR* + mutated *CALR*]) × 100. Patient 1 harbored a 5-base insertion (p.K385fs*47) and received peginterferon alfa-2a at a dose of 90 μg per week for the first 6 months and then 90 μg every other week. Patient 2 had a 52-base deletion (p.L637fs*46) and was treated with 180 μg of peginterferon alfa-2a every 2 weeks for the first year, followed by 180 μg every 3 weeks for the second year.

Cassinat B. Verger E,Kiladjian JJ. New Eng J Med 2015; 188-189

Interferon Alpha2 in MPNs

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- ✓ Perspectives

Interferon-Alpha

Mechanisms of Action

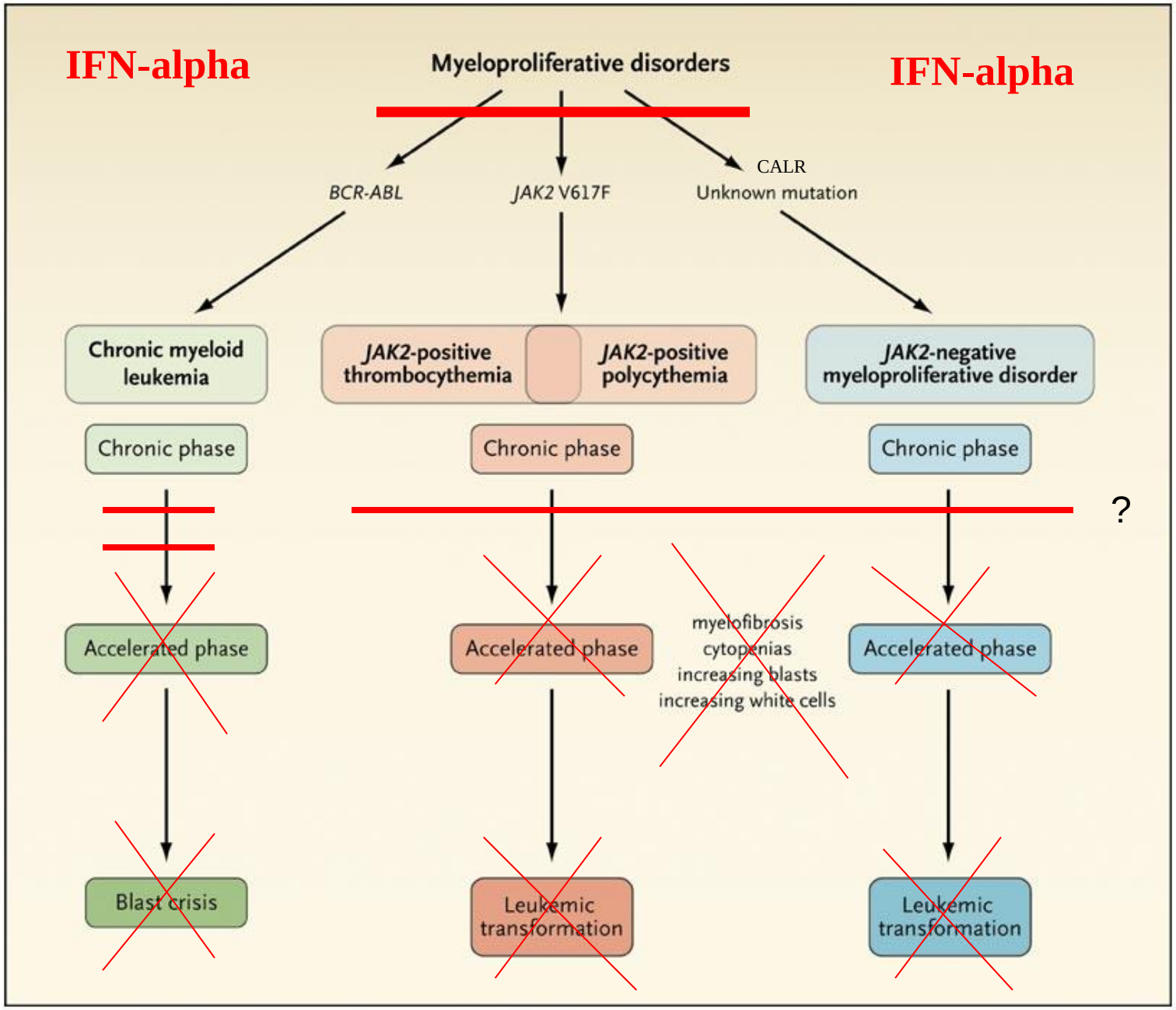
- ✓ Antiproliferative
- ✓ Proapoptotic
- ✓ Antiangiogenic
- ✓ **Immunoregulatory**
- ✓ Inhibition of telomerase

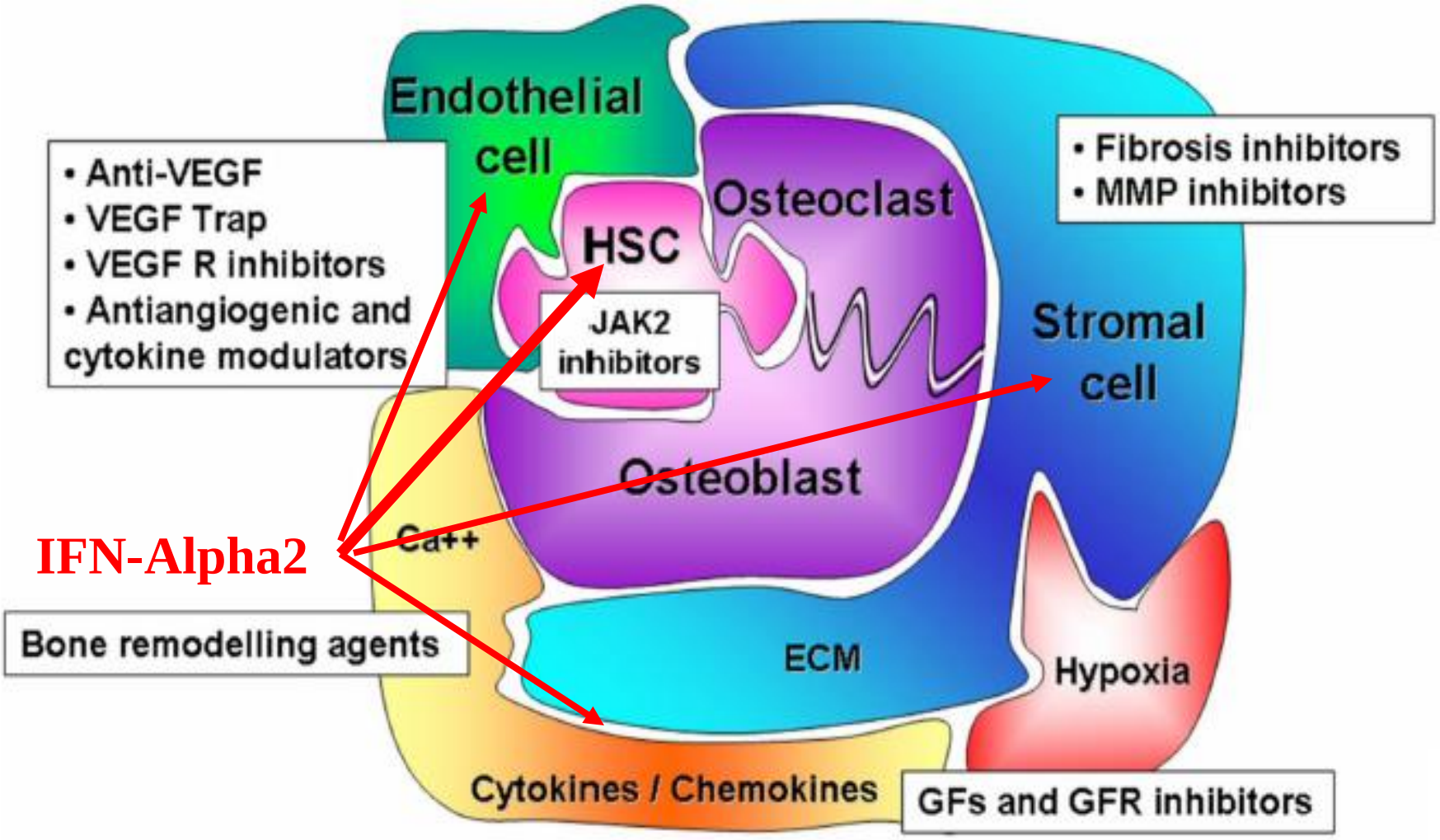
Interferon-Alpha

Immunoregulatory Activities

- ✓ Stimulate the cytotoxic activity of T-cells, NK-cells, monocytes, macrophages and DC
- ✓ Enhanced expression of anti-apoptotic genes in T-lymphocytes
- ✓ Increased expression of tumor-associated and HLA- antigens

Stem Cell Wake Up Call





Hematopoietic Niches: A Therapeutic Target for IFN ?

- ✓ **IFN-alpha2 wakes up dormant stem cells , put them in cycle and mobilize them to be targets for potent tumor killing**
- ✓ **IFN-alpha2 blocks the intramedullary release of cytokines from the bone marrow stroma**

Interferon Alpha2 in MPNs

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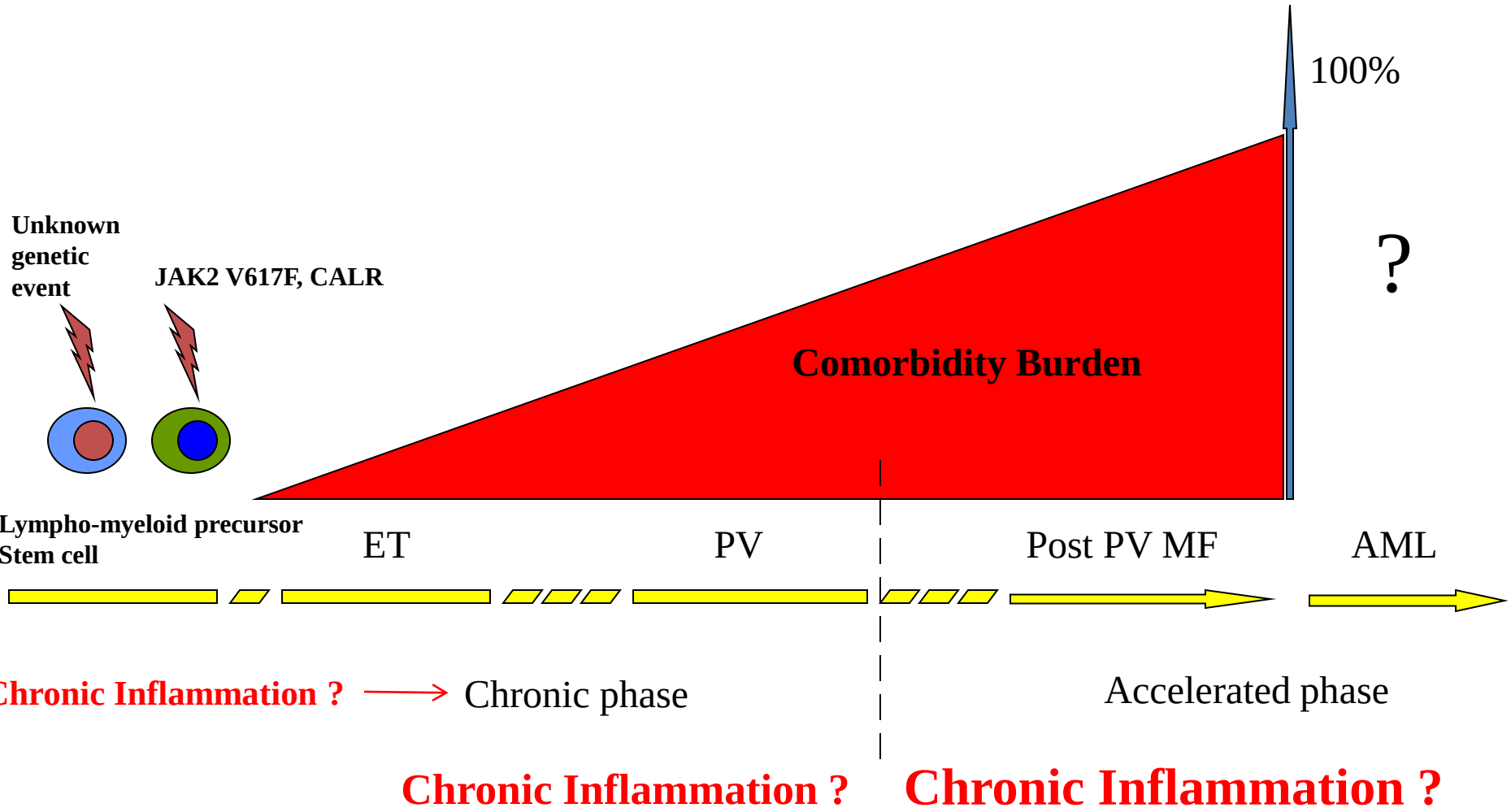
Tumor Burden and Comorbidity Burden in MPNs



MPNs

ET – PV - PMF

A Human Inflammation Model ?

A Human Cancer Model ?

Chronic Inflammation – Genomic Instability - Clonal Evolution ?

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Review

Chronic inflammation as a promotor of mutagenesis in essential thrombocythemia, polycythemia vera and myelofibrosis. A human inflammation model for cancer development?

Hans Carl Hasselbalch*

Oxidative Stress – ROS-Genomic Instability - Cancer

Department of Hematology, Roskilde Hospital, University of Copenhagen, Køgevej 7-13, Roskilde, Denmark

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Myelofibrosis

ABSTRACT

The Philadelphia-negative chronic myeloproliferative neoplasms (MPNs) are acquired stem cell neoplasms, in which a stem cell lesion induces an autonomous proliferative advantage. In addition to the JAK2V617 mutation several other mutations have been described. Recently chronic inflammation has been proposed as a trigger and driver of clonal evolution in MPNs. Herein, it is hypothesized that sustained inflammation may elicit the stem cell insult by inducing a state of chronic oxidative stress with elevated levels of reactive oxygen species (ROS) in the bone marrow, thereby creating a high-risk microenvironment for induction of mutations due to the persistent inflammation-induced oxidative damage to DNA in hematopoietic cells. Alterations in the epigenome induced by the chronic inflammatory drive may likely elicit a "epigenetic switch" promoting persistent inflammation. The perspectives of chronic inflammation as the driver of mutagenesis in MPNs is discussed, including early intervention with interferon-alpha2 and potent anti-inflammatory agents (e.g. JAK1-2 inhibitors, histone deacetylase inhibitors, DNA-hypomethylators and statins) to disrupt the self-perpetuating chronic inflammation state and accordingly eliminating a potential trigger of clonal evolution and disease progression with myelofibrotic and leukemic transformation.

Leukemia (2013), 1–9

www.nature.com/leu

ORIGINAL ARTICLE

A role for reactive oxygen species in *JAK2*^{V617F} myeloproliferative neoplasm progression

C Marty[1,2,3], C Lacout[1,2,3], N Droin[1,2,3], J-P Le Couédic[1,2,3], V Ribrag[1,2,3], E Solary[1,2,3], W Vainchenker[1,2,3], J-L Villeval[1,2,3] and I Plo[1,2,3]

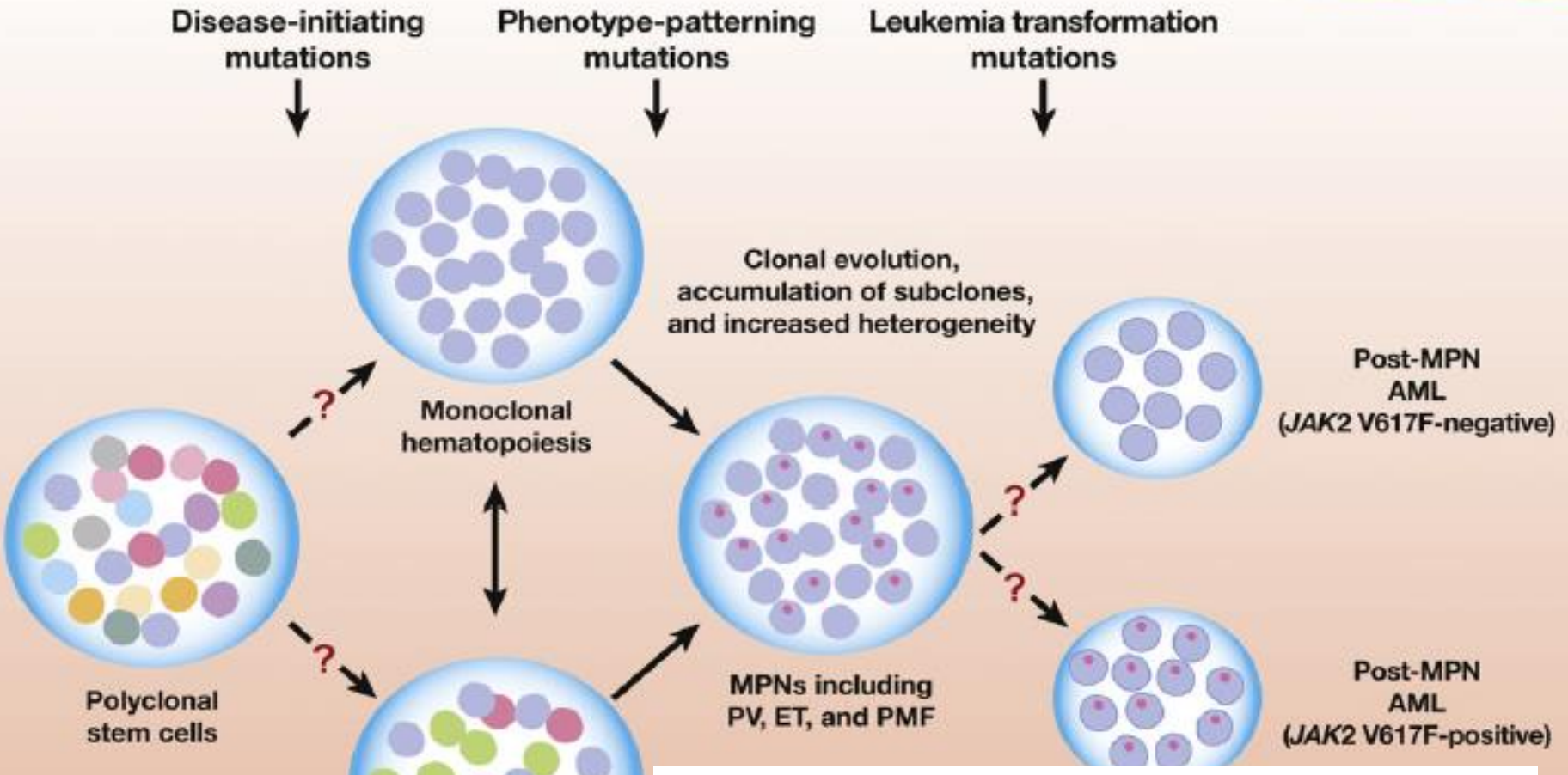
Although other mutations may predate the acquisition of the *JAK2*^{V617F} mutation, the latter is sufficient to drive the disease phenotype observed in BCR-ABL-negative myeloproliferative neoplasms (MPNs). One of the consequences of JAK2^{V617F} is genetic instability that could explain *JAK2*^{V617F}-mediated MPN progression and heterogeneity. Here, we show that JAK2^{V617F} induces the accumulation of reactive oxygen species (ROS) in the hematopoietic stem cell compartment of a knock-in (KI) mouse model and in patients with *JAK2*^{V617F} MPNs. JAK2^{V617F}-dependent ROS elevation was partly mediated by an AKT-induced decrease in catalase expression and was accompanied by an increased number of 8-oxo-guanines and DNA double-strand breaks (DSBs). Moreover, there was evidence for a mitotic recombination event in mice resulting in loss of heterozygosity of *Jak2*^{V617F}. Mice engrafted with 30% of *Jak2*^{V617F} KI bone marrow (BM) cells developed a polycythemia vera-like disorder. Treatment with the anti-oxidant N-acetylcysteine (NAC) substantially restored blood parameters and reduced damages to DNA. Furthermore, NAC induced a marked decrease in splenomegaly with reduction in the frequency of the *Jak2*^{V617F}-positive hematopoietic progenitors in BM and spleen. Altogether, overproduction of ROS is a mediator of JAK2^{V617F}-induced DNA damages that promote disease progression. Targeting ROS accumulation might prevent the development of *JAK2*^{V617F} MPNs.

Leukemia advance online publication, 26 April 2013; doi:10.1038/leu.2013.102

Keywords: myeloproliferative neoplasms; JAK2^{V617F}; reactive oxygen species; N-acetylcysteine; DNA damages; knock-in mouse model

Modified after Delhommeau et. al., 2010

Chronic Inflammation



- **JAK2V617F induces accumulation of ROS**
- **ROS induces DNA-damage in stem cells**
- **DNA-damages induce genomic instability**
- **Genomic instability induces mutations**

Marty C, Lacout C, Droin N et al. A role for reactive oxygen species in JAK2(V617F) myeloproliferative neoplasm progression. Leukemia. 2013 Apr 5.

Smoking is a Highly Potent Inflammation Stimulus

Smoking as a contributing factor for development of polycythemia vera and related neoplasms

Hans Carl Hasselbalch

Department of Hematology, Roskilde Hospital, University of Copenhagen, Koegevej 7-13, 4000 Roskilde, Denmark

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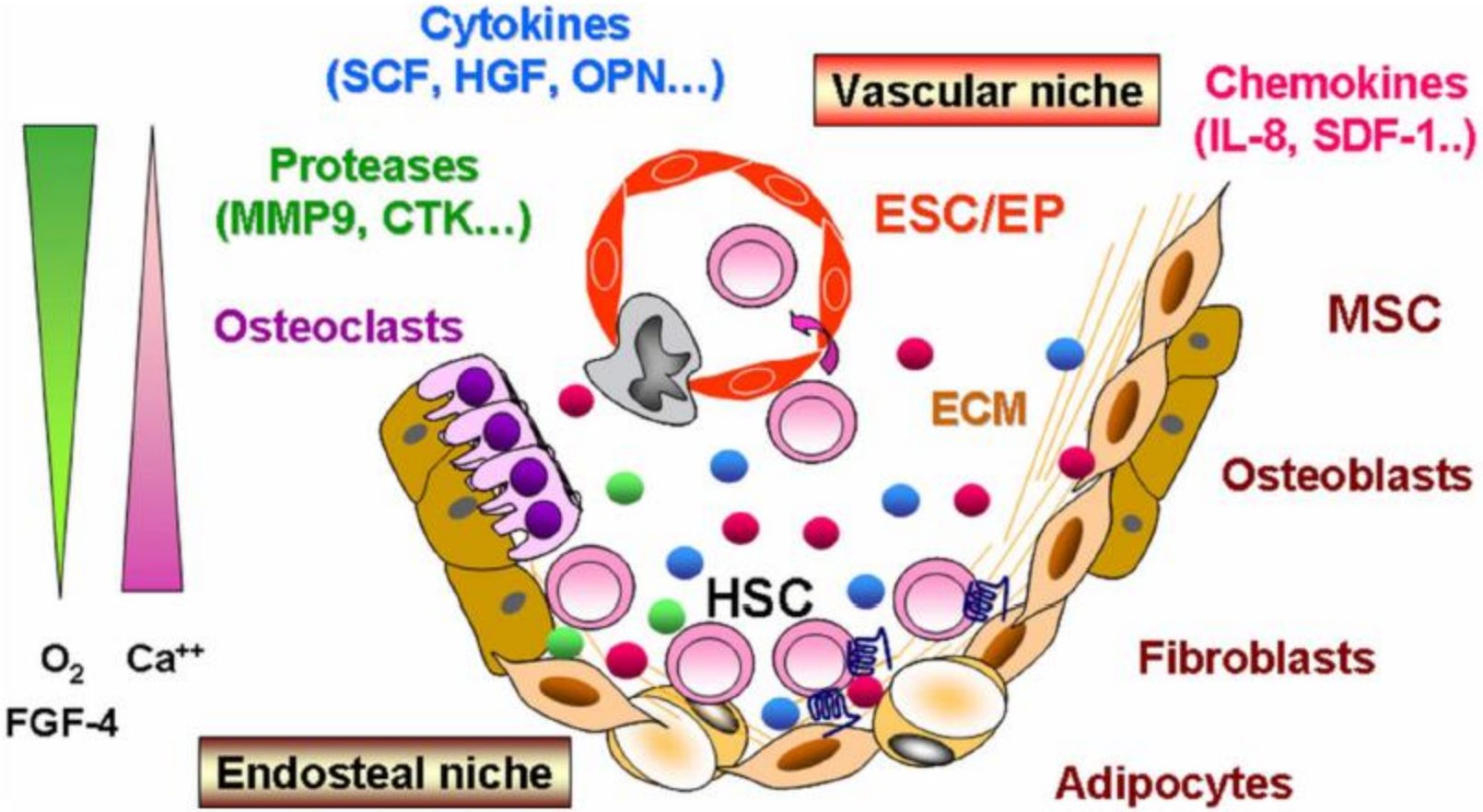
Myeloproliferative neoplasms MPNs
Thrombosis
Atherosclerosis
NF-κB
JAK-STAT-signaling
IL-8

ABSTRACT

Smoking may be associated with accelerated erythropoiesis, leukocytosis and thrombocytosis, which are also hallmarks in patients with polycythemia vera, essential thrombocythemia and early stages of myelofibrosis (MPNs). The JAK-STAT and NF-κB signaling pathways are activated in both smokers and in patients with MPNs. Additionally, both share elevated levels of several proinflammatory cytokines, in vivo activation of leukocytes and platelets, endothelial dysfunction and increased systemic oxidative stress. Based upon experimental, epidemiological and clinical data it is herein argued and discussed, if smoking may be involved in MPN pathogenesis, considering most recent studies and reviews which are supportive of the concept that chronic inflammation with NF-κB activation and oxidative stress may have a major role – both as triggers but also as the driving force for clonal expansion in MPNs.



A simplistic model of hematopoietic stem cell niches

Lataillade JJ, Pierre-Louis O, Hasselbalch HC , Uzan G, Jasmin C, Martyré MC, Le Bousse-Kerdilès MC; French INSERM and the European EUMNET Networks on Myelofibrosis. Does primary myelofibrosis involve a defective stem cell niche? From concept to evidence. Blood. 2008 15;112(8):3026-35.

An even more simplistic model of hematopoietic stem cell niches

The Inflamed Bone Marrow

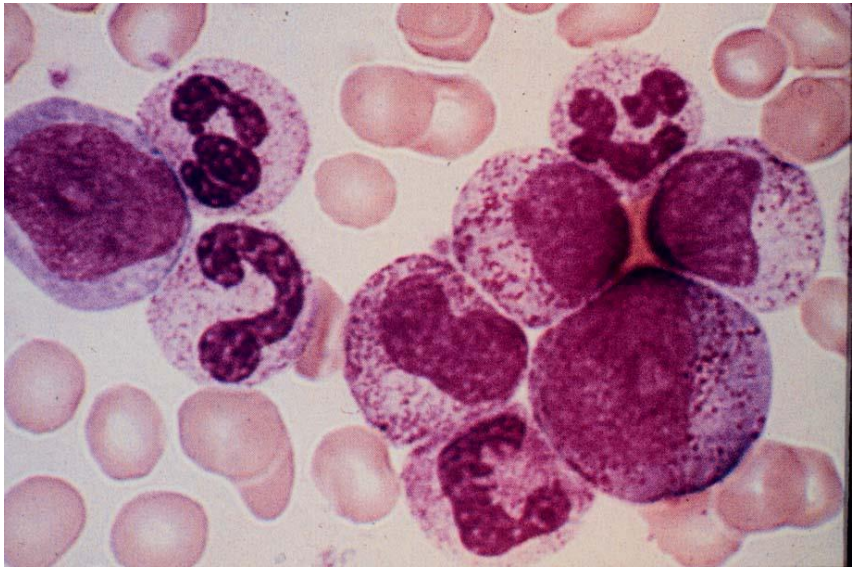
The chicks are flying prematurely (escaping) from the burning nest

Oxidative Stress – ROS Accumulation
Genomic Instability – Mutagenesis - Metastastis

Neutrophil Granules

MMM

- **M**obilization
- **M**etastasis
- **M**yeloid Metaplasia



OPEN ACCESS Freely available online

PLOS ONE

Transcriptional Profiling of Whole Blood Identifies a Unique 5-Gene Signature for Myelofibrosis and Imminent Myelofibrosis Transformation

Hans Carl Hasselbalch[1]*, Vibe Skov[2], Thomas Stauffer Larsen[3], Mads Thomassen[2], Caroline Hasselbalch Riley[4], Morten K. Jensen[4], Ole Weis Bjerrum[5], Torben A. Kruse[2]

1 Department of Hematology, Roskilde Hospital, University of Copenhagen, Roskilde, Denmark, **2** Department of Clinical Genetics, Odense University Hospital, Odense, Denmark, **3** Department of Hematology X, Odense University Hospital, Odense, Denmark, **4** Department of Hematology L, Herlev Hospital, University of Copenhagen, Herlev, Denmark, **5** Department of Hematology L, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

Abstract

Identifying a distinct gene signature for myelofibrosis may yield novel information of the genes, which are responsible for progression of essential thrombocythemia and polycythemia vera towards myelofibrosis. We aimed at identifying a simple gene signature – composed of a few genes - which were selectively and highly deregulated in myelofibrosis patients. Gene expression microarray studies have been performed on whole blood from 69 patients with myeloproliferative neoplasms. Amongst the top-20 of the most upregulated genes in PMF compared to controls, we identified 5 genes (DEFA4, ELA2, OLFM4, CTSG, and AZU1), which were highly significantly deregulated in PMF only. None of these genes were significantly regulated in ET and PV patients. However, hierarchical cluster analysis showed that these genes were also highly expressed in a subset of patients with ET (n = 1) and PV (n = 4) transforming towards myelofibrosis and/or being featured by an aggressive phenotype. We have identified a simple 5-gene signature, which is uniquely and highly significantly deregulated in patients in transitional stages of ET and PV towards myelofibrosis and in patients with PMF only. Some of these genes are considered to be responsible for the derangement of bone marrow stroma in myelofibrosis. Accordingly, this gene-signature may reflect key processes in the pathogenesis and pathophysiology of myelofibrosis development.

Citation: Hasselbalch HC, Skov V, Stauffer Larsen T, Thomassen M, Hasselbalch Riley C, et al. (2014) Transcriptional Profiling of Whole Blood Identifies a Unique 5-Gene Signature for Myelofibrosis and Imminent Myelofibrosis Transformation. PLoS ONE 9(1): e85567. doi:10.1371/journal.pone.0085567

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Competing Interests: The authors have declared that no competing interests exist.

* E-mail: hans.hasselbalch@dadlnet.dk

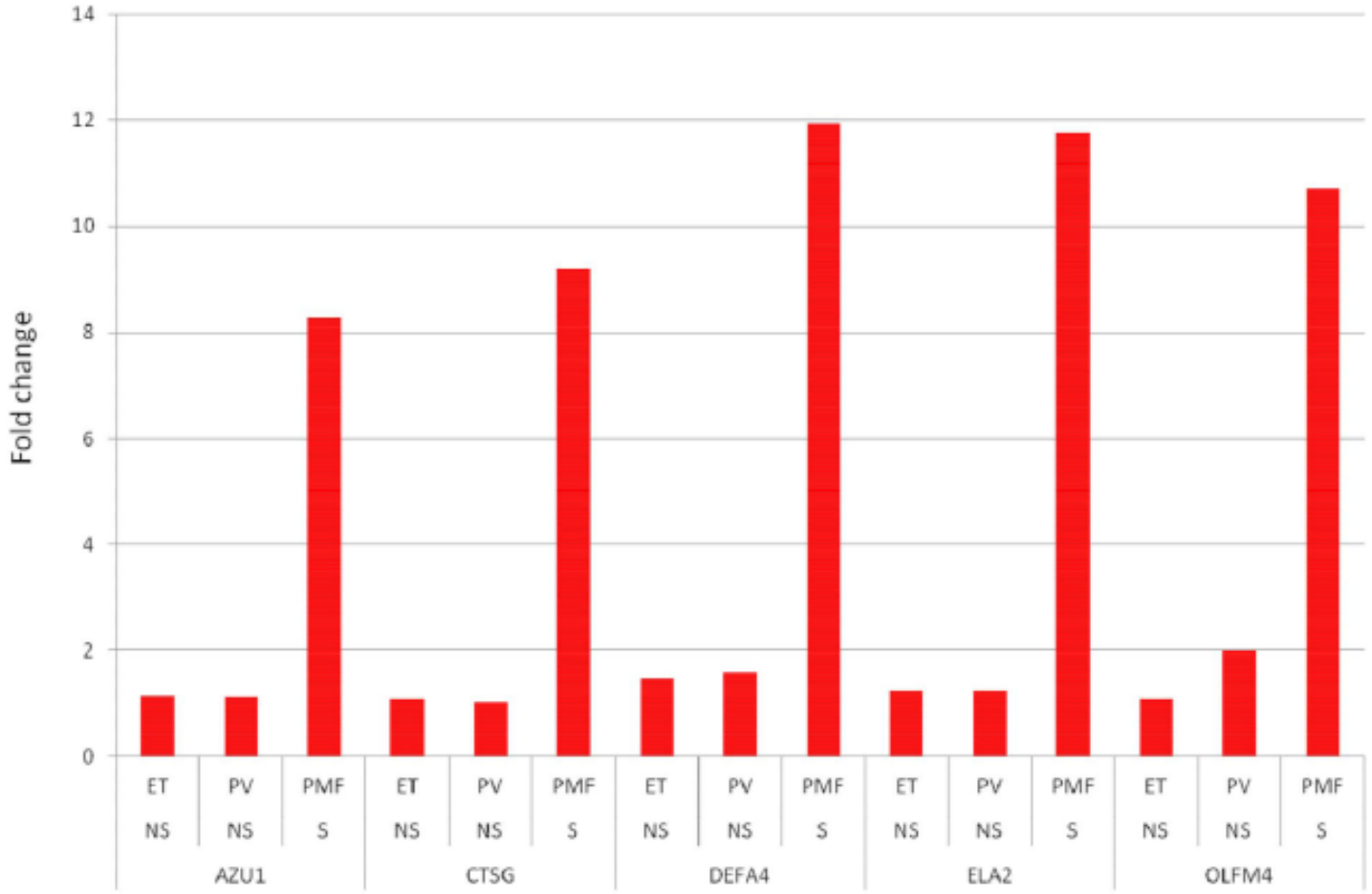
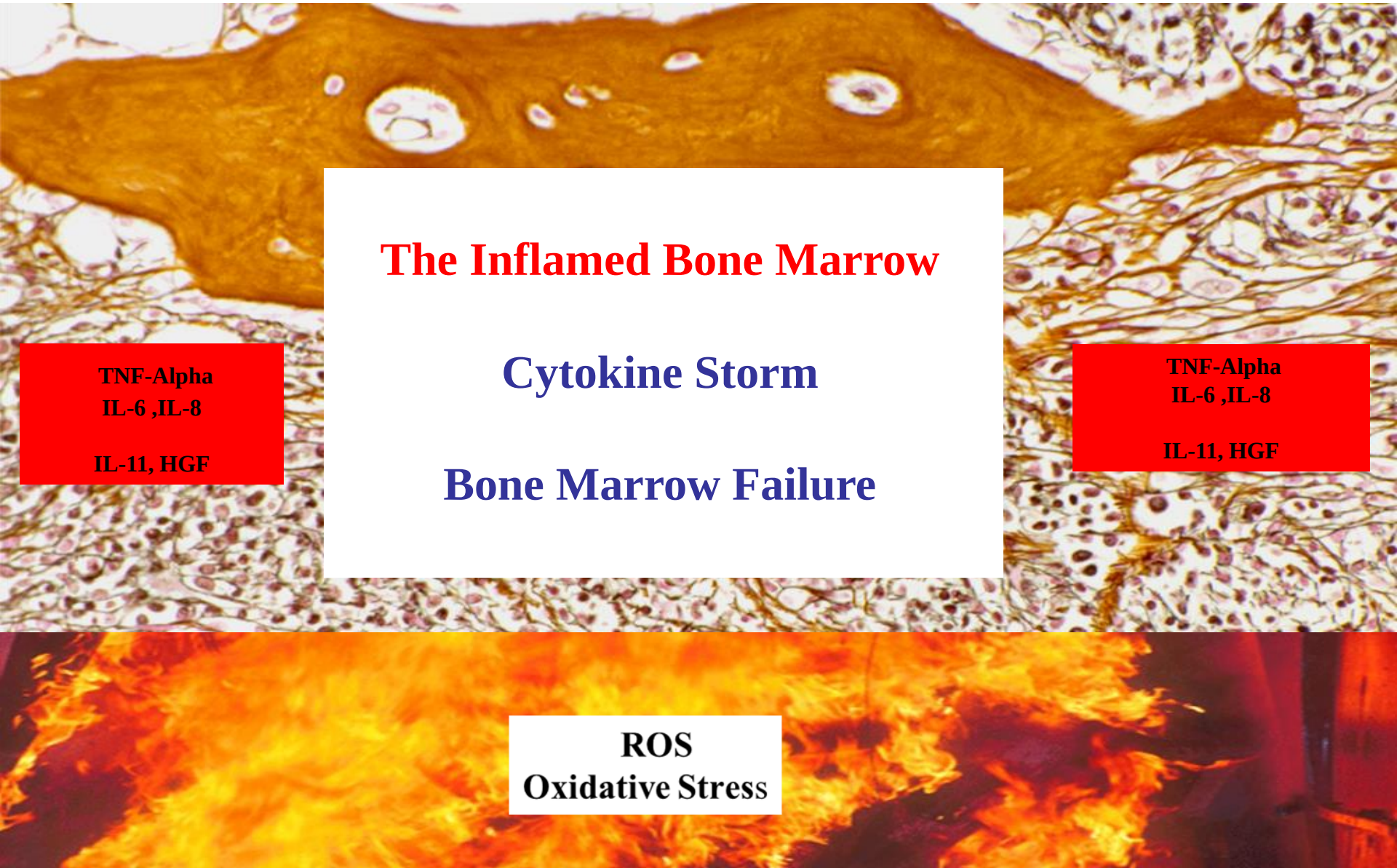


Figure 1. Fold changes for the 5 genes in ET, PV, and PMF compared to control subjects. Patient groups and genes are shown on the x-axis and fold changes on the Y-axis. NS: non-significant; S: significant. All genes FDR<0.05.
doi:10.1371/journal.pone.0085567.g001

Inflammation in The Bone Marrow



The Inflamed Bone Marrow

Cytokine Storm

Bone Marrow Failure

TNF-Alpha
IL-6 ,IL-8

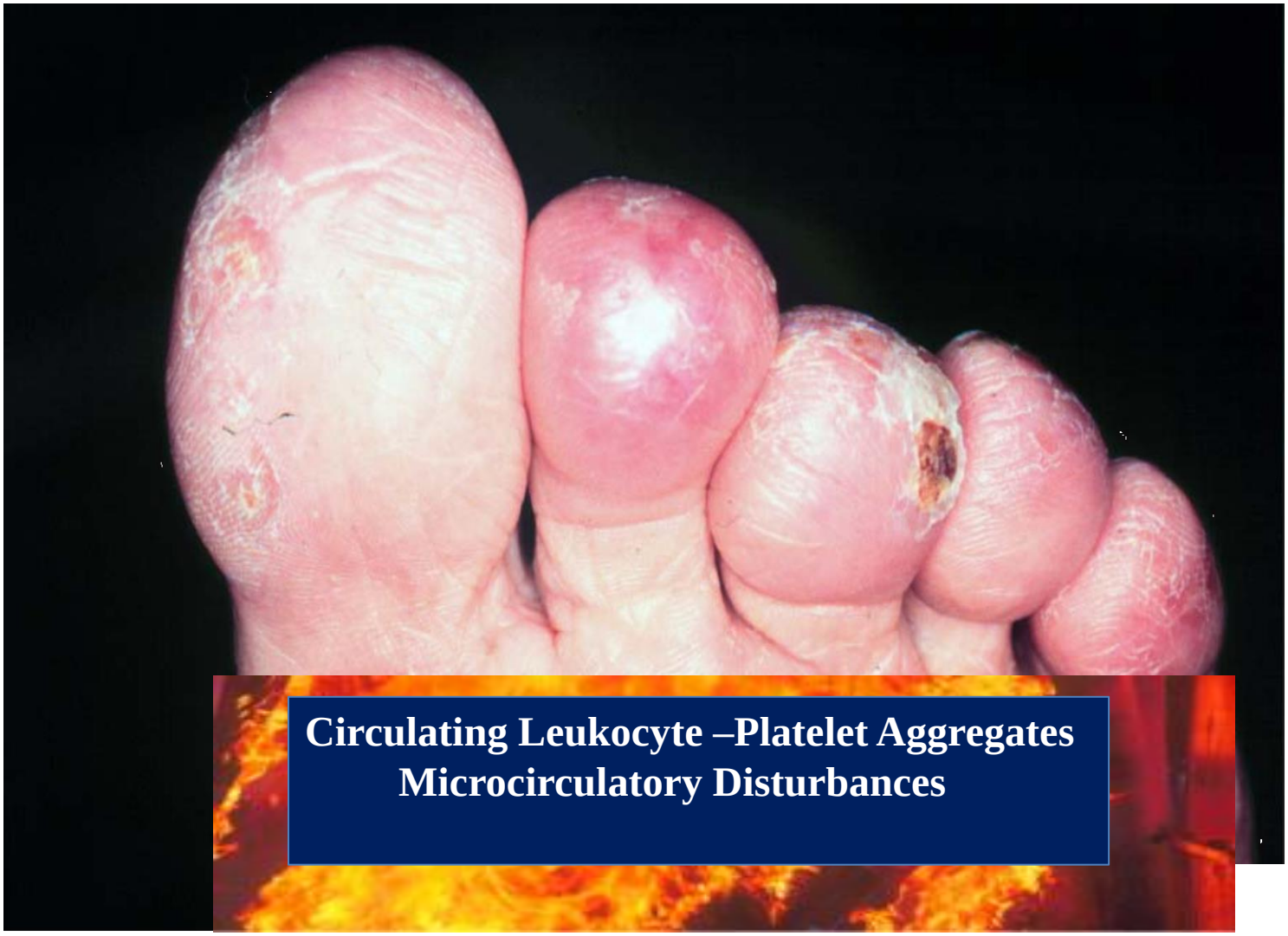
IL-11, HGF

TNF-Alpha
IL-6 ,IL-8

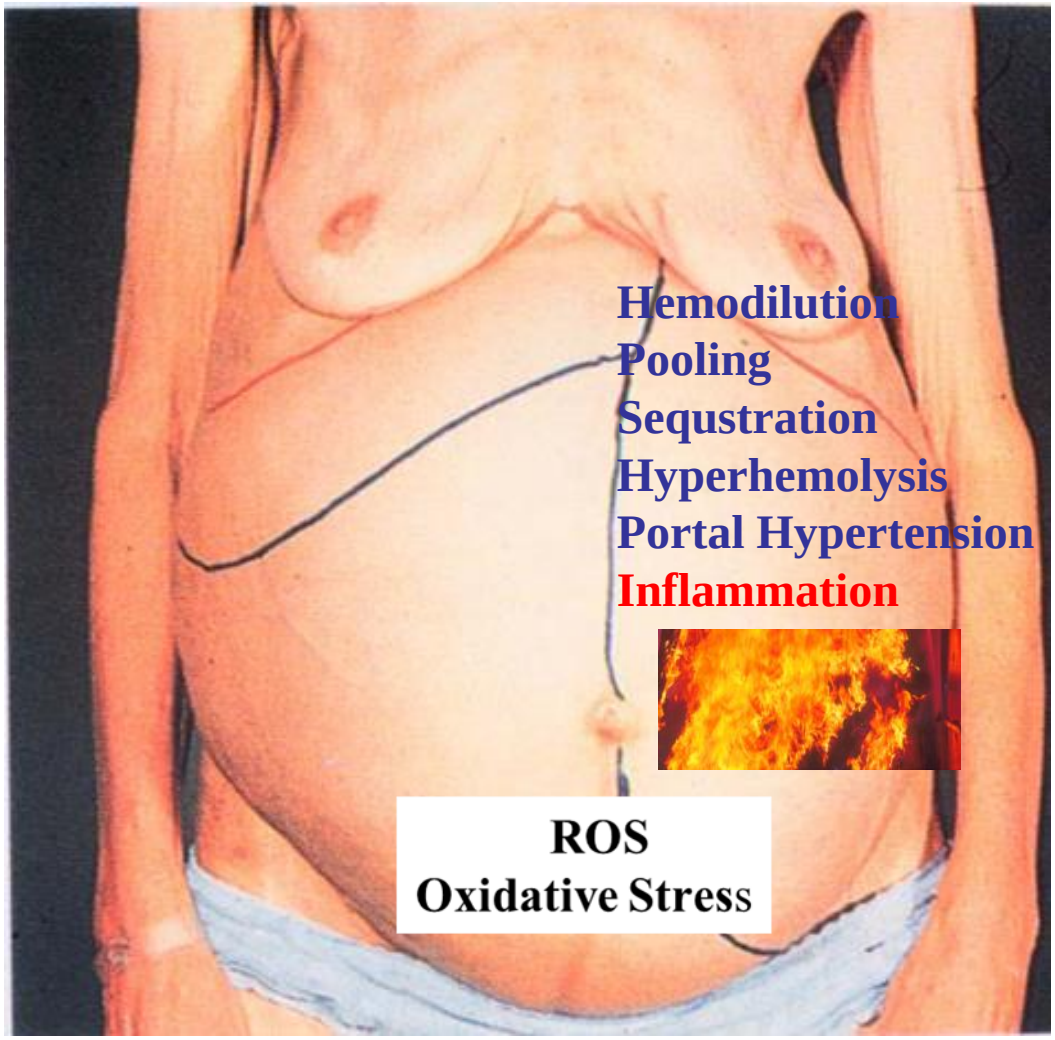
IL-11, HGF

ROS
Oxidative Stress

Inflammation in The Circulation



Inflammation in The Spleen



Myelofibrosis with huge splenomegaly

Anemia: bone marrow failure, hemodilution, pooling, sequestration, hyperhemolysis, portal hypertension, bleeding

Chronic Inflammation and Oxidative Stress

Clinical Implications ?

- Driver of clonal evolution , mutagenesis, subclone formation and myelofibrotic /leukemic transformation in MPNs ?

- Driver of development of premature atherosclerosis and early ageing ?

- Driver of development of other inflammation-mediated comorbidities , including second cancers ?

Chronic Inflammation and Oxidative Stress

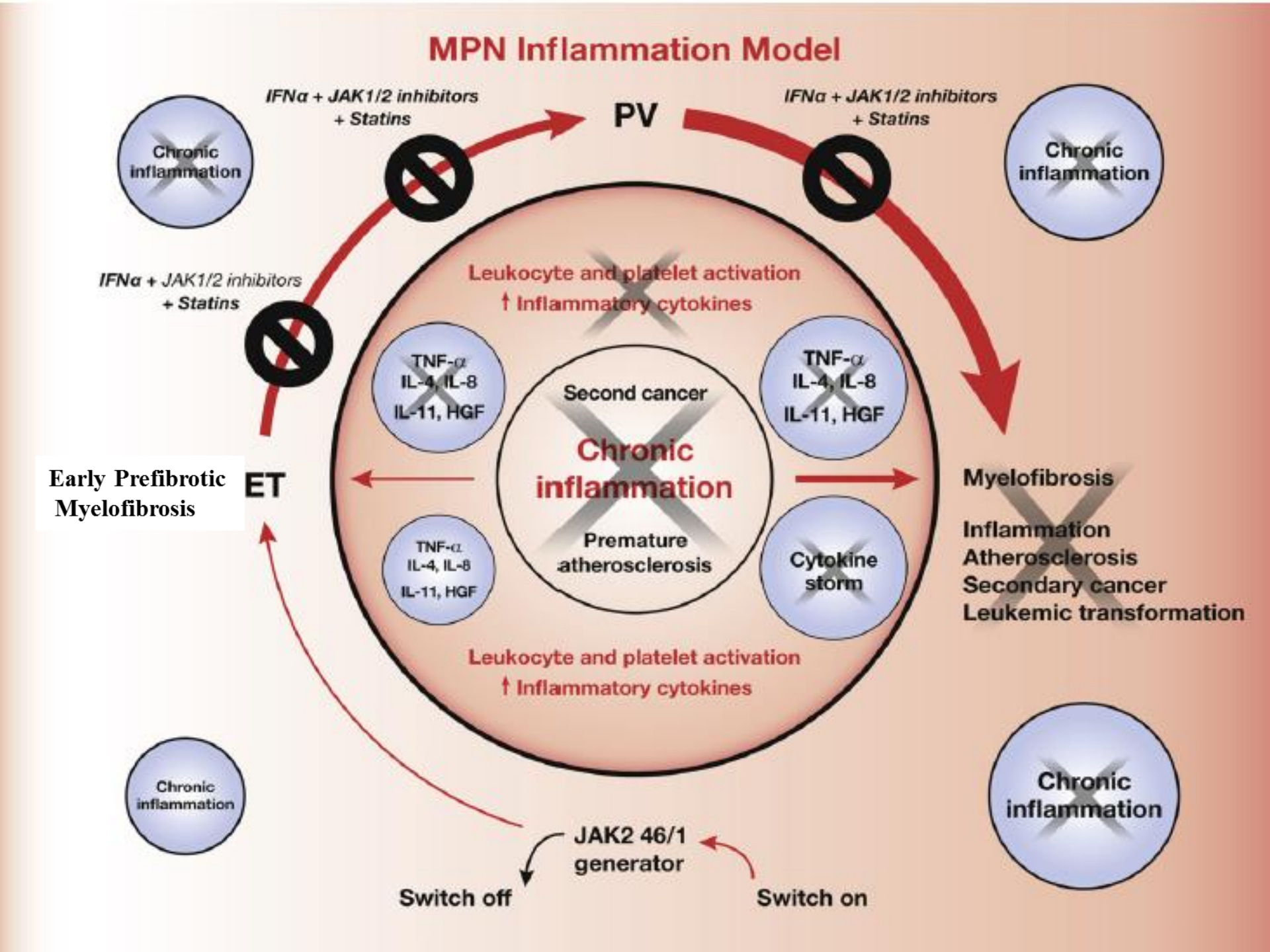
Therapeutic Implications ?

- Induction of resistance /refractoriness to treatment (eg. more hydroxyurea needed to control (inflammation-mediated) leuko-and thrombocytosis ?)'
- Impairment of IFN-signalling ?

How to Quell the Fire ?

- Early intervention when the chance of quelling the fire is the very best :

- STOP THE FUEL SUPPLY : Interferon-alpha
- ANTIINFLAMMATION : JAK1-2 inhibitor, statins ?


MPN Inflammation Model
IFNα + JAK1/2 inhibitors + Statins
PV
IFNα + JAK1/2 inhibitors + Statins
Chronic inflammation
Chronic inflammation
IFNα + JAK1/2 inhibitors + Statins
Leukocyte and platelet activation
↑ Inflammatory cytokines
TNF-α IL-4, IL-8 IL-11, HGF
TNF-α IL-4, IL-8 IL-11, HGF
Second cancer
Chronic inflammation
Premature atherosclerosis
TNF-α IL-4, IL-8 IL-11, HGF
Cytokine storm
Early Prefibrotic Myelofibrosis
ET
Myelofibrosis
Inflammation
Atherosclerosis
Secondary cancer
Leukemic transformation
Leukocyte and platelet activation
↑ Inflammatory cytokines
Chronic inflammation
Chronic inflammation
JAK2 46/1 generator
Switch off
Switch on

Two Different Scenarios

No Access IFN-alpha2	Access IFN-alpha2
• ”Do no harm”	• ~~”Do no harm”~~
• Risk stratification	• ~~Risk Stratification~~
• Normal blood counts	• Normal blood counts
• ~~Cytogenetic remission~~	• Cytogenetic remission
• ~~Molecular remission~~	• Molecular remission
• ~~Normal bone marrow~~	• Normal bone marrow
• ~~Minimal residual disease~~	• Minimal residual disease

Two Different Scenarios

No Access IFN-alpha2	Access IFN-alpha2
• STOP HU	• STOP IFN
Sustained	Sustained
• Complete HR • Molecular remission • Normal bone marrow • Minimal residual disease	• Complete HR • Molecular remission • Normal bone marrow • Minimal residual disease A subset of patients

Two Different Scenarios

No Access IFN-alpha2	Access IFN-alpha2
HU (>10 yrs)	**IFN-alpha2**
Risk of	Risk of
Skin cancer ?	~~Skin cancer~~ ?
MDS/AML ?	~~MDS/AML~~ ?
Second cancer ?	~~Second cancer~~ ?

Rationales for Early Intervention with IFN-Alpha2

- ✓ Major /Complete Molecular Remissions after Long-Term Treatment (> 3 -5 years)
- ✓ Sustained Molecular Remissions after Discontinuation of IFN-alpha2
- ✓ Minimal Residual Disease
- ✓ JAK2V617F ET : the Early Phase of PV in a Subset of Patients
- ✓ “ET “ Early Phase of Myelofibrosis in a Subset of Patients
- ✓ The JAK2V617F-mutation a thrombosis promoter

The Goal ?

„Minimal Residual Disease“
„Operational Cure „

Cure?

Sustained Molecular Response in Polycythemia Vera Treated with Interferon Alfa-2b

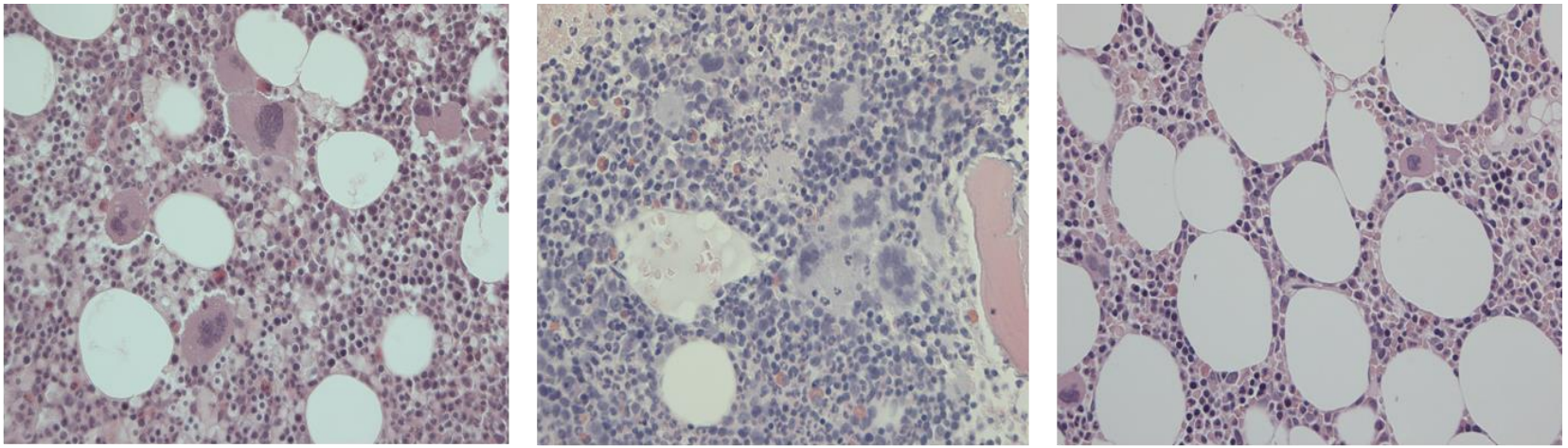
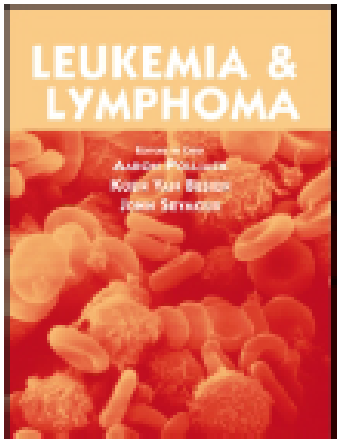


Figure 1: Bone marrow histomorphology from patient 1 at a) time of diagnosis 1996 and b) just prior to treatment with IFN alfa-2b. Both panels demonstrate classical PV features with hyperplasia and clustering of morphological abnormal megakaryocytes. Panel c) shows the morphologically normal bone marrow from August 2007 (after eight years of treatment with IFN-alfa 2b) with total regression of PV features (Larsen T et al Ann Hematol 2008; 87: 847–850)



Leukemia & Lymphoma

ISSN: 1042-8194 (Print) 1029-2403 (Online) Journal homepage: http://www.tandfonline.com/loi/ilal20

Minimal residual disease after long-term interferon-alpha2 treatment: a report on hematological, molecular and histomorphological response patterns in 10 patients with essential thrombocythemia and polycythemia vera

Cecilie Utke Rank, Ole Weis Bjerrum, Thomas Stauffer Larsen, Lasse Kjær, Karin de Stricker, Caroline Hasselbalch Riley & Hans Carl Hasselbalch

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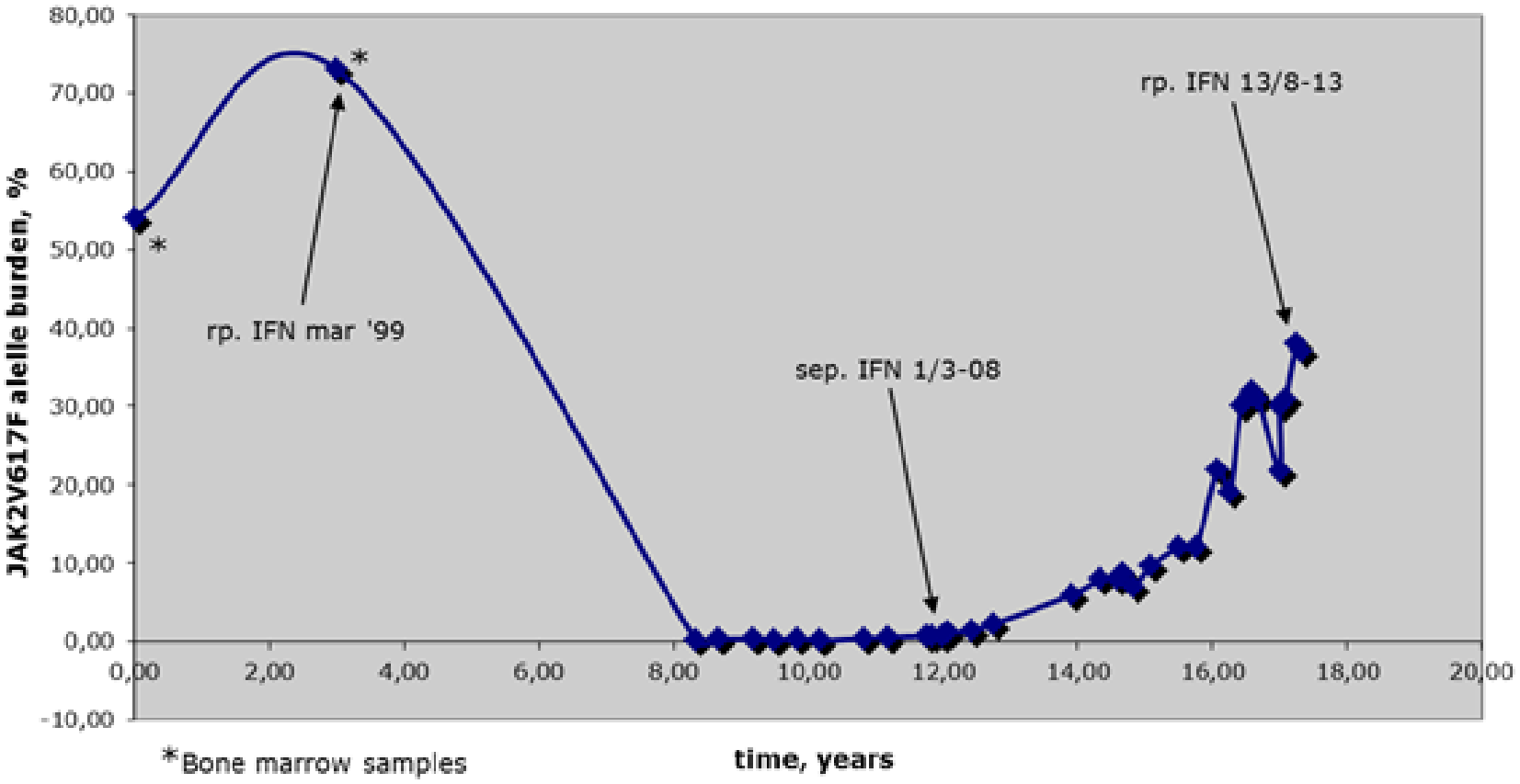
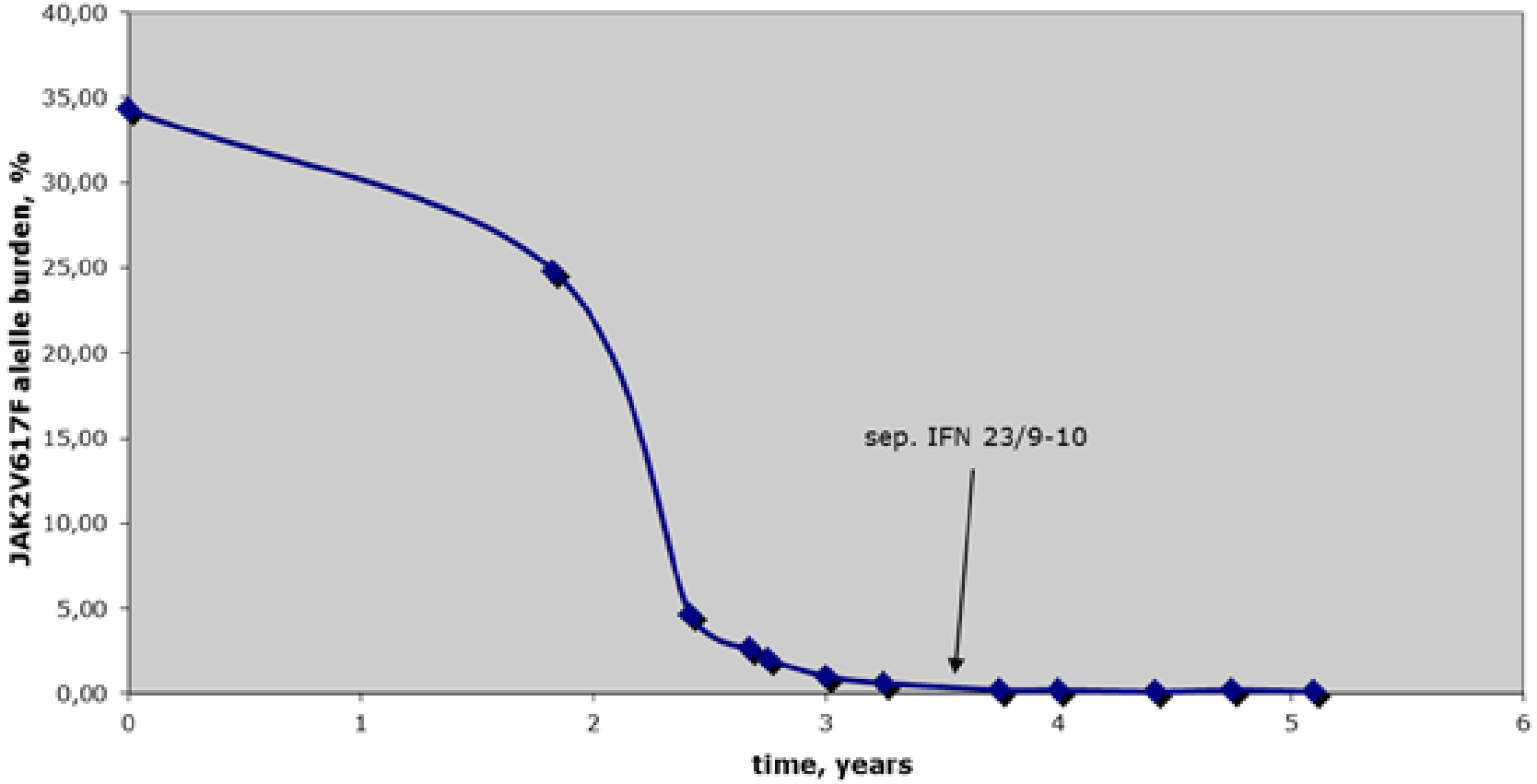
To link to this article: http://dx.doi.org/10.3109/10428194.2015.1049171

Patient 2: Serial Measurements of JAK2V617F during and after discontinuation of interferon-alpha treatment



Patient 9: Serial Measurements of JAK2V617F during and after discontinuation of interferon-alpha treatment



Impaired Tumor Immune Surveillance in MPNs ?

Chronic Inflammation ?

Immune Deregulation ?

Chronic myeloproliferative neoplasms and subsequent cancer risk: a Danish population-based cohort study

Henrik Frederiksen,[1,2] Dóra Körmendiné Farkas,[1] Christian Fynbo Christiansen,[1] Hans Carl Hasselbalch,[3] and Henrik Toft Sørensen[1]

[1]Department of Clinical Epidemiology, Aarhus University Hospital, Aarhus, Denmark; [2]Department of Hematology, Odense University Hospital, Odense, Denmark; and [3]Department of Hematology, Roskilde Hospital, Denmark

Patients with chronic myeloproliferative neoplasms, including essential thrombocythemia (ET), polycythemia vera (PV), and chronic myeloid leukemia (CML), are at increased risk of new hematologic malignancies, but their risk of nonhematologic malignancies remains unknown. In the present study, we assessed the risk of both types of malignancies after an ET, PV, or CML diagnosis. We linked 2 population-based nationwide registries, the Danish National Registry of Patients, covering all Danish hospitals and the Danish Cancer Registry, and assessed subsequent cancer risk in a cohort of all 7229 patients diagnosed with a chronic myeloproliferative neoplasm during 1977-2008. We compared the incidence of subsequent cancer in this cohort with that expected on the basis of cancer incidence in the general population (standardized incidence ratio). Overall, ET, PV, and CML patients were at increased risk of developing both new hematologic and nonhematologic cancers. The standardized incidence ratio for developing a nonhematologic cancer was 1.2 (95% confidence interval [95% CI]): 1.0-1.4) for patients with ET, 1.4 (95% CI: 1.3-1.5) for patients with PV, and 1.6 (95% CI: 1.3-2.0) for patients with CML. We conclude that patients with chronic myeloproliferative neoplasms are at increased risk of developing a new malignant disease. (*Blood*. 2011;118(25):6515-6520)

EUROPEAN JOURNAL OF Haematology

European Journal of Haematology

ORIGINAL ARTICLE

Increased incidence of another cancer in myeloproliferative neoplasms patients at the time of diagnosis

Helna Pettersson[1], Håvar Knutsen[2], Erik Holmberg[3], Björn Andréasson[1,4]

[1]Hematology section, Uddevalla, NU hospital group, Uddevalla, Sweden; [2]Hematology section, Ullevål Hospital, Oslo, Norway; [3]Regional Oncology Center, Göteborg; [4]Section of Hematology and Coagulation, Sahlgrenska University Hospital, Göteborg, Sweden

Abstract

Several studies have reported an increased incidence of coexistent cancer in patients with myeloproliferative neoplasms (MPN), and myelosuppressive treatment has been speculated to be one of the causes. In this study, we have concentrated on malignancies diagnosed before the MPN diagnosis to eliminate the possible influence of MPN treatment. The patients were recruited from the Swedish and Norwegian cancer registries. One thousand seven hundred and 45 patients from the Swedish MPN Quality Registry and 468 patients from the Norwegian National Cancer Registry were included in this study covering a 3-yr period. The results show that primary concurrent cancer is higher among patients with MPN compared to the general population. When pooled together, the Swedish and the Norwegian cohort showed increased prevalence of all types of cancer in general compared with the general population, standard prevalence ratio (SPR) of 1.20 (95% CI 1.07–1.34). Significantly high SPRs were reached for skin malignant melanoma [1.89 (95% CI 1.33–2.62)], prostate cancer [1.39 (95% CI 1.11–1.71)], and hematologic cancer [1.49 (95% CI 1.00–2.12)]. In the polycythemia vera group, the risk of having prior malignant melanoma of the skin was significant, with an SPR of 2.20 (95% CI 1.17–3.77). For patients with essential thrombocythemia and primary myelofibrosis, no significant risks were found. Coexisting cancers have a high impact on the treatment strategies of MPN, as it narrows down the treatment options. Chronic inflammation, as a common denominator of MPN with other cancers, can catalyze each other's existence and progression.

Key words myeloproliferative neoplasm; polycythemia vera; essential thrombocythemia; primary myelofibrosis; cancer

Correspondence Björn Andréasson, Section of Hematology and Coagulation, Sahlgrenska University Hospital, 41380 Göteborg, Sweden. Tel: +46104352100; Fax: +46104357017; e-mail: bjorn.andreasson@vgregion.se

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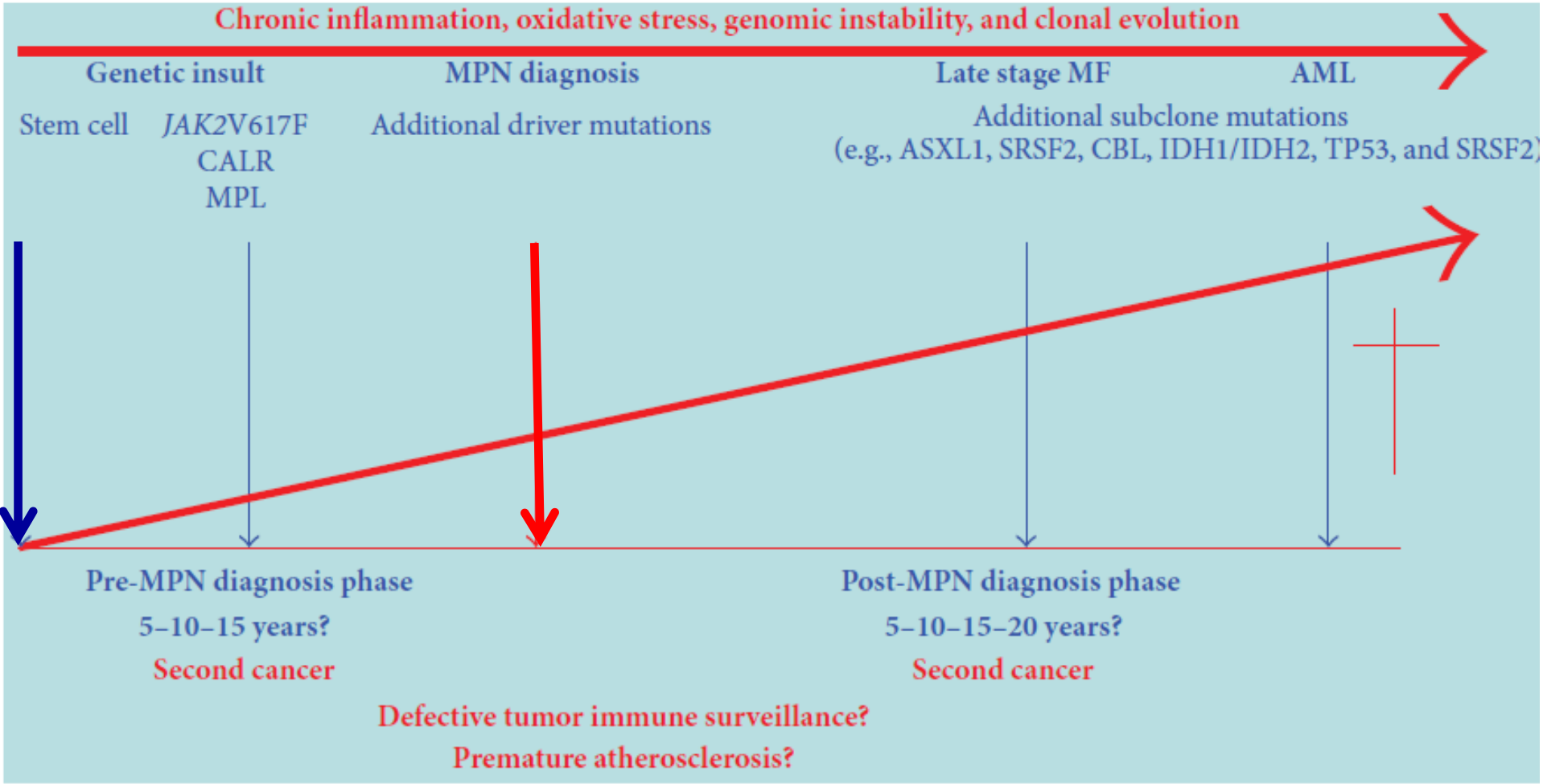
Chronic Inflammation and Second Cancer in MPNs



Leukemia & Lymphoma, October 2013; 54(10): 2269–2273

ISSN: 1042-8194 print / 1029-2403 online
DOI: 10.3109/10428194.2013.764417

informa
healthcare

ORIGINAL ARTICLE: RESEARCH

Whole blood transcriptional profiling reveals significant down-regulation of human leukocyte antigen class I and II genes in essential thrombocythemia, polycythemia vera and myelofibrosis

Vibe Skov[1], Caroline Hasselbalch Riley[2], Mads Thomassen[1], Thomas Stauffer Larsen[3], Morten K. Jensen[2], Ole Weis Bjerrum[4], Torben A. Kruse[1] & Hans Carl Hasselbalch[5]

Perspectives :

- Down-regulation of HLA-genes is a ”tumor-escape mechanism” by which tumor cells escape the attack from potent immune cells e.g. cytotocic T cells and NK-cells)
- Interferon-alpha2 potently upregulate HLA-genes on tumor cells thereby rendering them accessible for tumor killing by IFN-alpha2
- Early treatment with IFN to enhance tumor cell killing

Interferon Alpha2 in MPNs

- ✓ History of Interferons
- ✓ Biology and Mechanisms of Action
- ✓ The Biological Continuum (ET-PV-MF)
- ✓ The Novel Concept of Chronic Inflammation in MPNs
- ✓ Early Intervention Concept
- ✓ Minimal Residual Disease
- ✓ **Interferon Resistance and Intolerability**
- ✓ Perspectives

Interferon Resistance

J Interferon Res. 1994 Aug;14(4):187-9.

Interferon antibodies in thrombocythemia.

Merup M, Engman K, Paul C.

Am J Hematol. 1995 Mar;48(3):163-7.

Alpha-2a interferon therapy and antibody formation in patients with essential thrombocythemia and polycythemia vera with thrombocytosis.

Törnebohm-Roche E, Merup M, Lockner D, Paul C.

Author information

Abstract

In ten patients with essential thrombocythemia and polycythemia vera with thrombocytosis we have investigated the therapeutic effect of recombinant alpha-2a interferon (Roceron-A) given subcutaneously in a maintenance dosage of 3 million units three times weekly. The aim was to normalize the platelet count (< or = 400 x 10(9)/L). One of the secondary aims was to study platelet activity measured as beta-thromboglobulin (beta-TG) in urine. All but one patient could administer the injections and in all patients a significant reduction in platelet values was seen. The treatment was discontinued in three patients due to side effects of interferon, two because of hair loss (one with irreversible alopecia), and one because of depression. Three patients developed antibodies to alpha-2a interferon and a concomitant rise in the platelet level; in one patient therapy was switched to leukocyte alpha-interferon with an excellent response. The initial levels of beta-TG were elevated in 9/10 patients and were significantly reduced at 6 months in 4/5 patients not developing antibodies. Six patients are still on alpha-interferon therapy with a long-term follow-up of 3-3.5 years. We conclude that alpha-interferon therapy may be an alternative in patients with thrombocytosis and/or complications necessitating treatment.

Interferon Resistance

Buxhofer-Ausch V, Gisslinger H, Berg T, Gisslinger B, Kralovics R.

Acquired resistance to interferon alpha therapy associated with homozygous MPL-W515L mutation and chromosome 20q deletion in primary myelofibrosis.

Eur J Haematol. 2009 Feb;82(2):161-3.

The Molecular Heterogeneity of MPN is Complex

Variability in Molecular Responses to IFN-alpha

- TET2 clones persist in some patients
- Additional clones may impair the response to IFN-alpha2

Regular Article

CLINICAL TRIALS AND OBSERVATIONS

Molecular analysis of patients with polycythemia vera or essential thrombocythemia receiving pegylated interferon α-2a

Alfonso Quintás-Cardama,[1] Omar Abdel-Wahab,[2] Taghi Manshouri,[1] Outi Kilpivaara,[2,3] Jorge Cortes,[1] Anne-Laure Roupie,[2] Su-Jiang Zhang,[4] David Harris,[1] Zeev Estrov,[1] Hagop Kantarjian,[1] Ross L. Levine,[2] and Srdan Verstovsek[1]

[1]Department of Leukemia, MD Anderson Cancer Center, Houston, TX; [2]Human Oncology and Pathogenesis Program and Leukemia Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY; [3]Department of Medical Genetics, Genome-Scale Biology Research Program, University of Helsinki, Helsinki, Finland; and [4]Department of Hematology, The First Affiliated Hospital of Nanjing Medical University, Jiangsu Province Hospital, Nanjing, China

Key Points

- Treatment with PEG-IFN-α-2a in PV and ET results in a high rate of complete hematologic and molecular responses.
- Patients failing to achieve complete molecular remission tended to have higher frequencies of mutations in genes other than *JAK2*.

Pegylated interferon α-2a (PEG-IFN-α-2a) has previously been shown to induce hematologic and molecular responses in patients with polycythemia vera (PV) or essential thrombocythemia (ET). Here we present a follow-up of a phase 2 trial with PEG-IFN-α-2a treatment in 43 PV and 40 ET patients with detailed molecular analysis. After a median follow-up of 42 months, complete hematologic response was achieved in 76% of patients with PV and 77% of those with ET. This was accompanied by complete molecular response (CMR) (ie, undetectable *JAK2*V617F) in 18% and 17%, of PV and ET patients, respectively. Serial sequencing of *TET2*, *ASXL1*, *EZH2*, *DNMT3A*, and *IDH1/2* revealed that patients failing to achieve CMR had a higher frequency of mutations outside the Janus kinase–signal transducer and activator of transcription pathway and were more likely to acquire new mutations during therapy. Patients with both *JAK2*V617F and *TET2* mutations at therapy onset had a higher *JAK2*V617F mutant allele burden and a less significant reduction in *JAK2*V617F allele burden compared with *JAK2*mutant/*TET2*wild-type patients. These data demonstrate that PEG-IFN-α-2a induces sustained CMR in a subset of PV or ET patients, and that genotypic context may influence clinical and molecular response to PEG-IFN-α-2a. (*Blood*. 2013;122(6):893-901)

RESEARCH ARTICLE

AJH

Molecular responses and chromosomal aberrations in patients with polycythemia vera treated with peg-proline-interferon alpha-2b

Nicole C.C. Them,[1] Klaudia Bagienski,[1] Tiina Berg,[1] Bettina Gisslinger,[2] Martin Schalling,[2] Doris Chen,[1] Veronika Buxhofer-Ausch,[3,4] Josef Thaler,[5] Ernst Schloegl,[6] Guenther A. Gastl,[7] Dominik Wolf,[7,8] Karin Strecker,[3] Alexander Egle,[9] Thomas Melchardt,[9] Sonja Burgstaller,[5] Ella Willenbacher,[7] Oleh Zagrijtschuk,[10] Christoph Klade,[10] Richard Greil,[9] Heinz Gisslinger,[2] and Robert Kralovics[1,2]*

Fifty-one polycythemia vera (PV) patients were enrolled in the phase I/II clinical study PEGINVERA to receive a new formulation of pegylated interferon alpha (peg-proline-IFNα-2b, AOP2014/P1101). Peg-proline-IFNα-2b treatment led to high response rates on both hematologic and molecular levels. Hematologic and molecular responses were achieved for 46 and 18 patients (90 and 35% of the whole cohort), respectively. Although interferon alpha (IFNα) is known to be an effective antineoplastic therapy for a long time, it is currently not well understood which genetic alterations influence therapeutic outcomes. Apart from somatic changes in specific genes, large chromosomal aberrations could impact responses to IFNα. Therefore, we evaluated the interplay of cytogenetic changes and IFNα responses in the PEGINVERA cohort. We performed high-resolution SNP microarrays to analyze chromosomal aberrations prior and during peg-proline-IFNα-2b therapy. Similar numbers and types of chromosomal aberrations in responding and non-responding patients were observed, suggesting that peg-proline-IFNα-2b responses are achieved independently of chromosomal aberrations. Furthermore, complete cytogenetic remissions were accomplished in three patients, of which two showed more than one chromosomal aberration. These results imply that peg-proline-IFNα-2b therapy is an effective drug for PV patients, possibly including patients with complex cytogenetic changes.

Am. J. Hematol. 90:288–294, 2015.

- **High hematological and molecular responses (48 (90%)and 18 (35%) patients, respect.)**
- **Responses achieved independently of chromosomal aberrations**
- **Complete cytogenetic remissions in three patients**

Them N er al . Am J Hematol 90; 288-294,2015

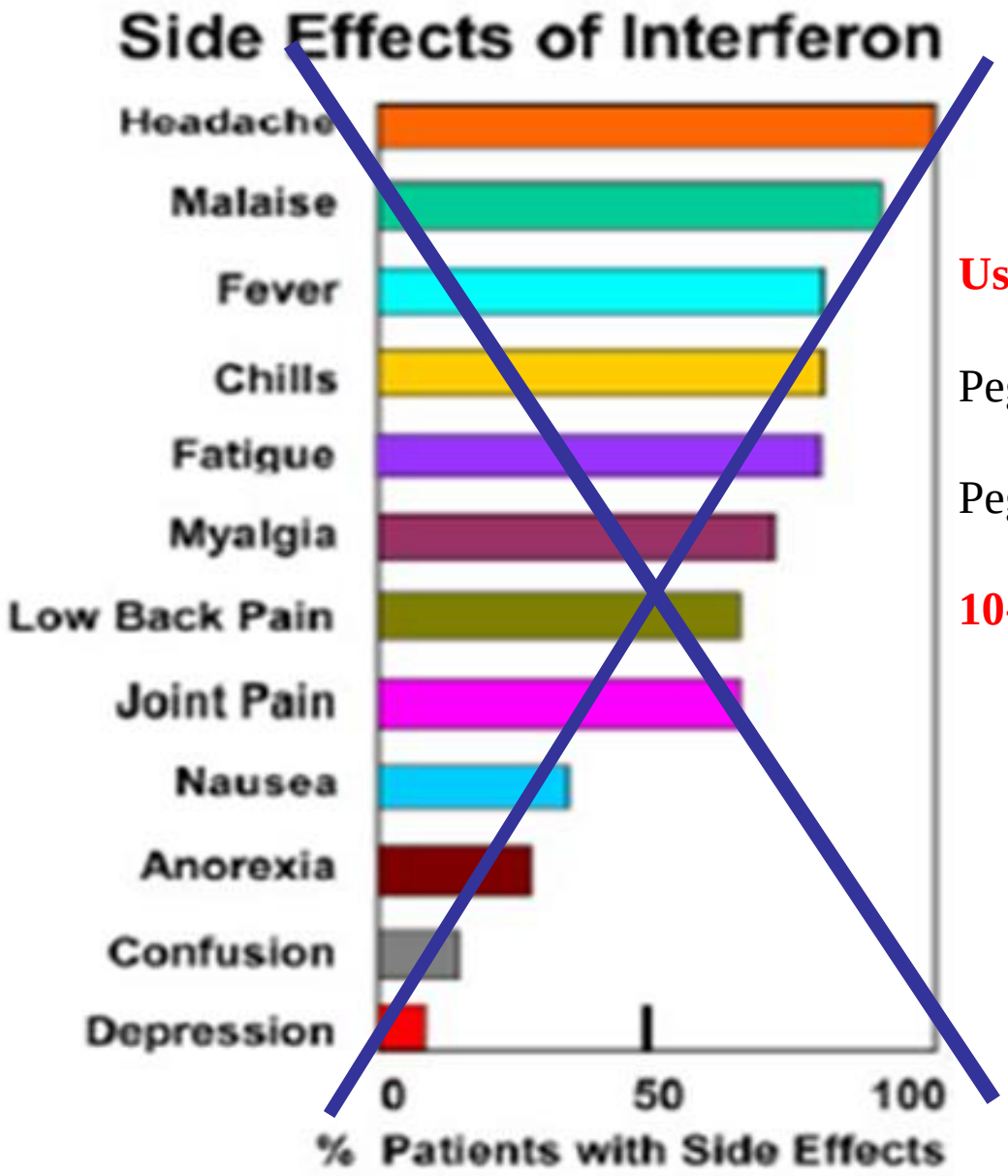
DALIAH

A Danish Study of Low-Dose Interferon-alpha2 versus Hydroxyurea in Ph-Negative Myeloproliferative Cancer

A National Multicenter Study on The Efficacy, Toxicity and QoL

200 Patients Included

Data Analysis Ongoing



Use Low Dose :

Pegasys 45 ug x 1 sc/week

PegIntron 30 ug x 1 sc/week

10-20 % side effects

Toxicity – Side Effects - Autoimmunity

Response Patterns

- the subgroup of patients with severe side effects (drop out) - a better and more rapid reponse to IFN ?

- The subgroup of patients with autoimmunity during treatment with IFN – a better and more rapid response to IFN ?

Combination Therapy

Interferon Alpha2 + JAK Inhibitor in Polycythemia Vera and Myelofibrosis

Leukemia Research Reports 3 (2014) 73–75

Contents lists available at ScienceDirect

Leukemia Research Reports

journal homepage: www.elsevier.com/locate/lrr

Case report

Combination therapy with interferon and JAK1-2 inhibitor is feasible: Proof of concept with rapid reduction in *JAK2*V617F-allele burden in polycythemia vera

M.E. Bjørn [a,*], K. de Stricker [b], L. Kjær [a], K. Ellemann [c], H.C. Hasselbalch [a]

[a] Department of Hematology, Roskilde University Hospital, Køgevej 7-13, 4000 Roskilde, Denmark
[b] Department of Pathology, Odense University Hospital, Sdr. Boulevard 29, 5000 Odense, Denmark
[c] Department of Neurology, Roskilde University Hospital, Køgevej 7-13, 4000 Roskilde, Denmark

ARTICLE INFO

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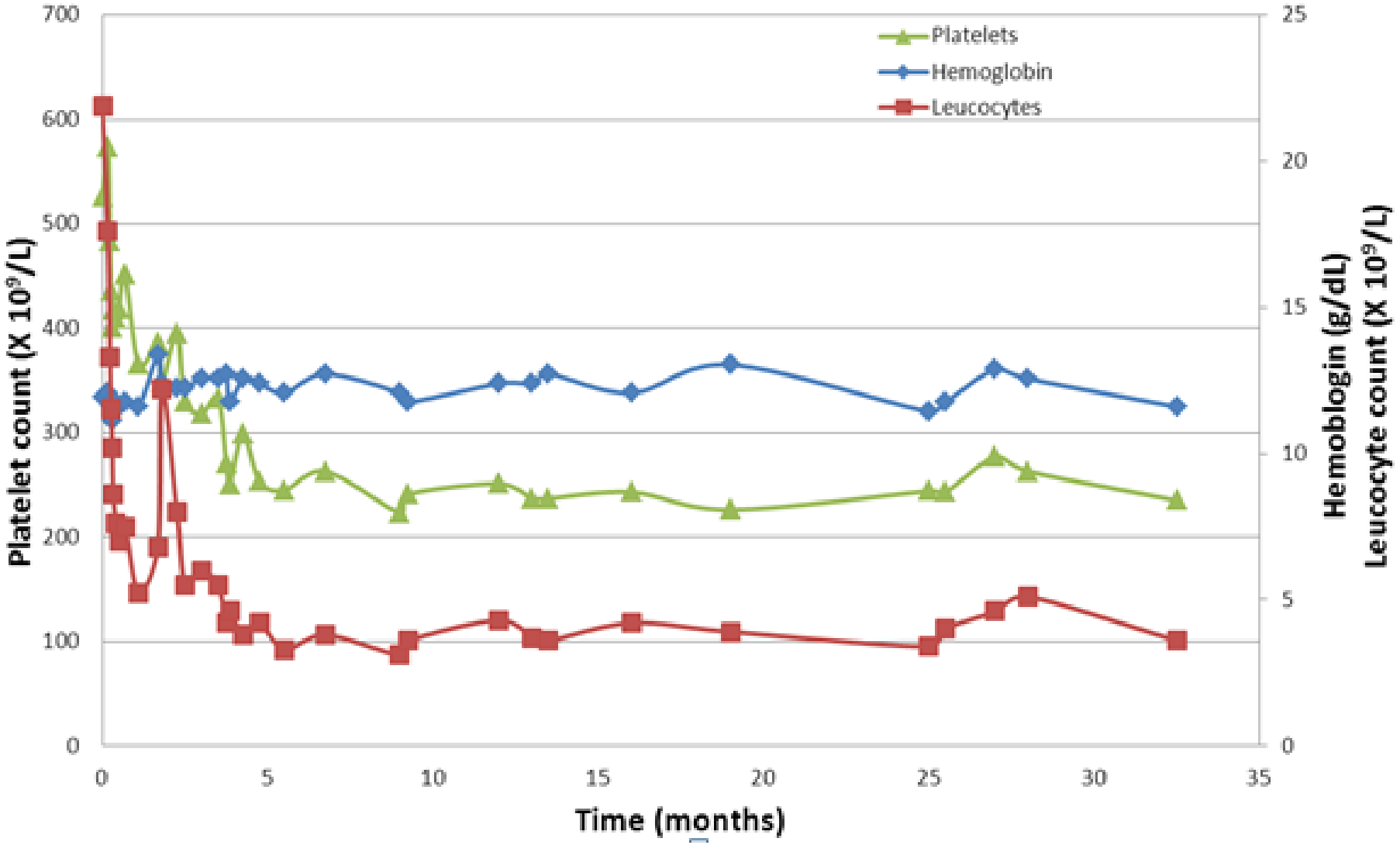
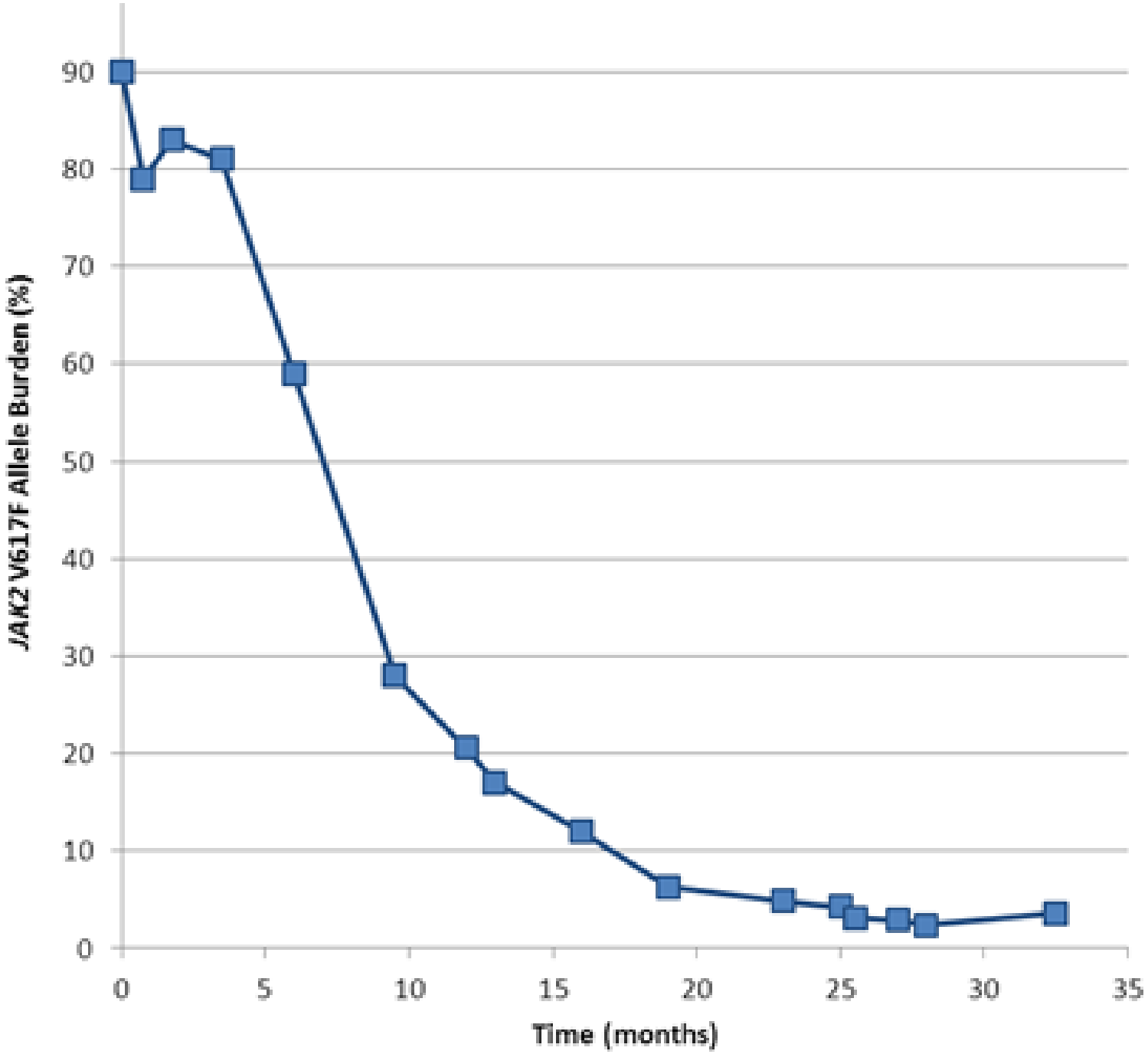
Polycythemia vera
*JAK2*V617F-allele burden
Combination therapy
Interferon
Ruxolitinib

ABSTRACT

We report a 55 year old woman with post-ET PV for 12 years, who experienced resolution of severe constitutional symptoms within 3 days, a marked reduction in splenomegaly and a rapid decline in the *JAK2*V617F allele burden during combination therapy with interferon-alpha2a and ruxolitinib. Within 4 weeks the patient achieved complete hematological remission with normalization of peripheral blood counts and within 10 months the *JAK2*V617F-allele burden was reduced from 90% to 28%. Such a rapid decline in the *JAK2*V617F allele burden is highly unusual in PV-patients during low-dose IFN-alpha2 monotherapy and this finding warrants a prospective study with combination therapy.


Platelets
Hemoglobin
Leucocytes
Platelet count (X 10⁹/L)
Hemoblogin (g/dL)
Leucocyte count (X 10⁹/L)
Time (months)
0
100
200
300
400
500
600
700
0
5
10
15
20
25
0
5
10
15
20
25
30
35


JAK2 V617F Allele Burden (%)
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
Time (months)

Safety and Efficacy of Combination Therapy of Interferon-Alpha2 + JAK1-2 Inhibitor in the Philadelphia-Negative Chronic Myeloproliferative Neoplasms

Preliminary Results from the Danish Combi-Trial - an Open Label, Single Arm, Non-Randomized Multicenter Phase II Study

Stine Ulrik Mikkelsen[1], Lasse Kjær[1], Vibe Skov[1], Mads Emil Bjørn[1], Christen Lykkegaard Andersen[2], Ole Weis Bjerrum[2], Nana Brochmann[1], Daniel El Fassi[3], Torben A Kruse[4], Thomas Stauffer Larsen[4], Torben Mourits-Andersen[5], Claus Henrik Nielsen[2], Niels Pallisgaard[1], Mads Thomassen[4] and Hans Carl Hasselbalch[1]

[1]Roskilde University Hospital, Roskilde, Denmark; [2]Rigshospitalet, University Hospital of Copenhagen, Copenhagen, Denmark; [3]Herlev University Hospital, Copenhagen, Denmark; [4]Odense University Hospital, Odense, Denmark; [5]Sydvestjysk Hospital, Esbjerg, Denmark

Conclusions

- Combination therapy with IFNa2 and ruxolitinib is highly efficacious in patients with PV or hyperproliferative MF
- Combination therapy was generally well tolerated without any unexpected toxicities
- The results of this interim analysis suggest that combination therapy with IFNa2 and ruxolitinib may be a promising new treatment option in MPN patients

MPNs and Inflammation

Targeting The Malignant Clone and Inflammation

- ✓ **Both IFNα and JAK2 inhibitors reduce the high levels of inflammatory cytokines that may be responsible for disease initiation and progression; accordingly, there is a strong rationale for a combination of the two substance classes**
- ✓ **This combination may result in a mutual augmentation of effects, leading to an increased efficacy compared with single-agent therapy.**
- ✓ **Combination therapy might allow the use of lower doses of each agent**
- ✓ **Ultimately, the question arises of whether this combination may exhibit a disease-modifying or curative effect**
- ✓ **The ability of IFNα to induce molecular responses and the role of inflammation in the initiation and progression of MPNs undoubtedly make such a combination therapy one of the most promising new strategies in the management of MPNs**

Koschmieder S, Mughal TI, Hasselbalch HC et al. Myeloproliferative neoplasms and inflammation: whether to target the malignant clone or the inflammatory process or both. Leukemia. 2016 ; 30(5):1018-24.

ELSEVIER

Journal of Hepatology 45 (2006) 271–279

Journal of Hepatology

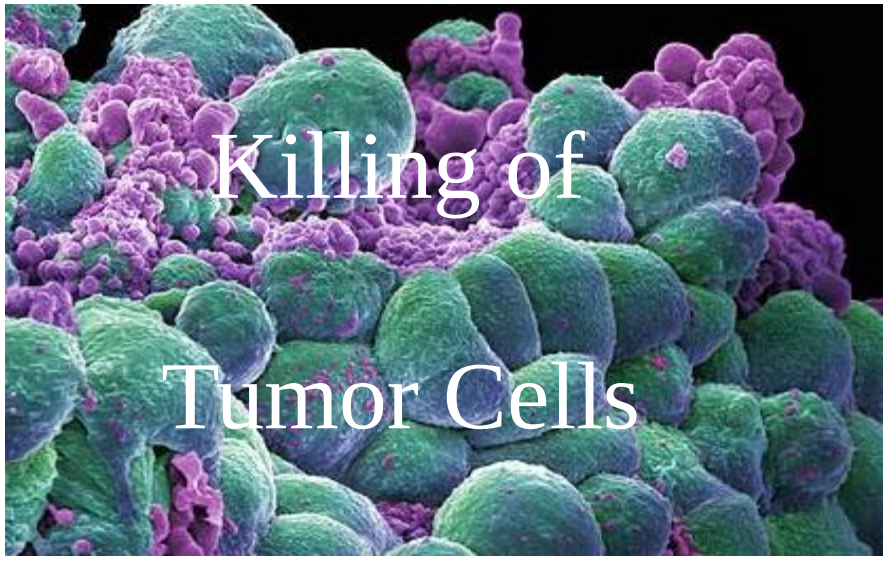
www.elsevier.com/locate/jhep

Oxidative stress inhibits IFN-α-induced antiviral gene expression by blocking the JAK–STAT pathway

Danilo Di Bona[1,2,*], Marco Cippitelli[3,4], Cinzia Fionda[3,4,†], Calogero Cammà[1,2], Anna Licata[1], Angela Santoni[3,4], Antonio Craxì[1]

[1] *Cattedra e Unità Operativa di Gastroenterologia, Dipartimento Biomedico e di Medicina Specialistica, University of Palermo, Palermo, Italy*
[2] *IBIM, Consiglio Nazionale delle Ricerche, Palermo, Italy*
[3] *Department of Experimental Medicine and Pathology, Istituto Pasteur-Fondazione Cenci Bolognetti, University 'La Sapienza', Rome, Italy*
[4] *Regina Elena Cancer Institute, Rome, Italy*


Oxidative Stress
Enhanced
Tumor Immune Surveillance


Killing of
Tumor Cells


T-Cell
T-Cell
NK-Cells
T-Cell
Statins
JAK2-Inhibitor
Interferon-Alpha

JAK1-2 Inhibition + Statins

Quelling the Fire

The Inflamed Bone Marrow

Statins and Anti-inflammation

- Inhibit leukocyte activation
- Inhibit platelet activation

- Inhibit release of pro-inflammatory cytokines (eg. IL-6, TNF-alfa)

Statins inhibit JAK2V617F-dependent cell growth

Statins enhance JAK2 inhibition

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Statin therapy improves response to interferon alfa and ribavirin in chronic hepatitis C: A systematic review and meta-analysis

Qianqian Zhu [a], Na Li [a], Qunying Han [a], Pingping Zhang [a], Cuiling Yang [a], Xiaoyan Zeng [a], Yanping Chen [a], Yi Lv [b,c], Xi Liu [a], Zhengwen Liu [a,c,*]

[a] Department of Infectious Diseases, First Affiliated Hospital, School of Medicine, Xi'an Jiaotong University, Xi'an 710061, Shaanxi, China
[b] Department of Hepatobiliary Surgery, First Affiliated Hospital, School of Medicine, Xi'an Jiaotong University, Xi'an 710061, Shaanxi, China
[c] Institute of Advanced Surgical Technology and Engineering, Xi'an Jiaotong University, Xi'an 710061, Shaanxi, China

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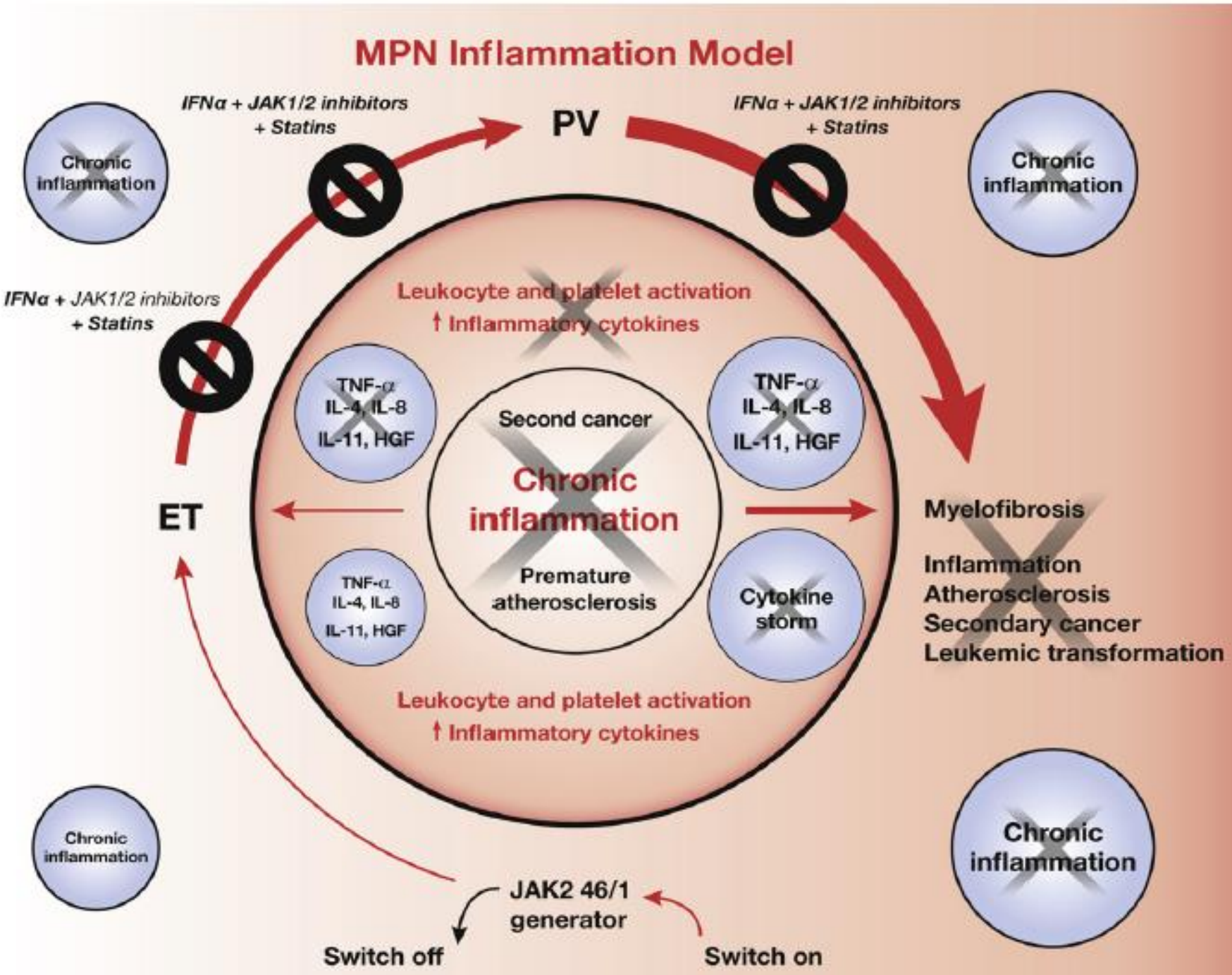
Chronic hepatitis C
Treatment
Statin
Interferon alfa
Ribavirin

ABSTRACT

The treatment of interferon alfa (IFN-α) and ribavirin for chronic hepatitis C virus (HCV) infection achieves limited sustained virological response (SVR). We conducted a systematic review and meta-analysis to explore the efficacy of adding statins to IFN-α and ribavirin therapy for chronic hepatitis C. Studies with data pertinent to the effect of statins on chronic hepatitis C were reviewed, and randomized controlled trials (RCTs) evaluating the efficacy of the addition of statins to IFN-α and ribavirin were included in meta-analysis. The primary outcome measure was SVR. Secondary outcome measures were rapid virological response (RVR) and early virological response (EVR). The literature was systematically searched through October 2012. After screening of the 1724 non-duplicated entries, 54 potentially relevant studies were fully reviewed. Of those, 18 studies were relevant and 5 RCTs met the inclusion criteria for meta-analysis. In comparison with IFN-α and ribavirin therapy, the addition of statins significantly increased SVR (OR = 2.02, 95% CI: 1.38–2.94), RVR (OR = 3.51, 95% CI: 1.08–11.42) and EVR (OR = 1.89, 95% CI: 1.20–2.98). The SVR increase remained significant for HCV genotype 1 (OR = 2.11, 95% CI: 1.40–3.18). There were no significant increases in adverse events and withdrawals with the addition of statins. In conclusion, the addition of statins to IFN-α and ribavirin improves SVR, RVR, and EVR without additional adverse events and thus may be considered as adjuvant to IFN-α and ribavirin for chronic hepatitis C. Statins might also be used for HCV genotypes other than genotype 1, or in patients in whom the use of protease inhibitors is contraindicated or not indicated.

Combination Therapy

Interferon Alpha2 + Ruxolitinib + Statin

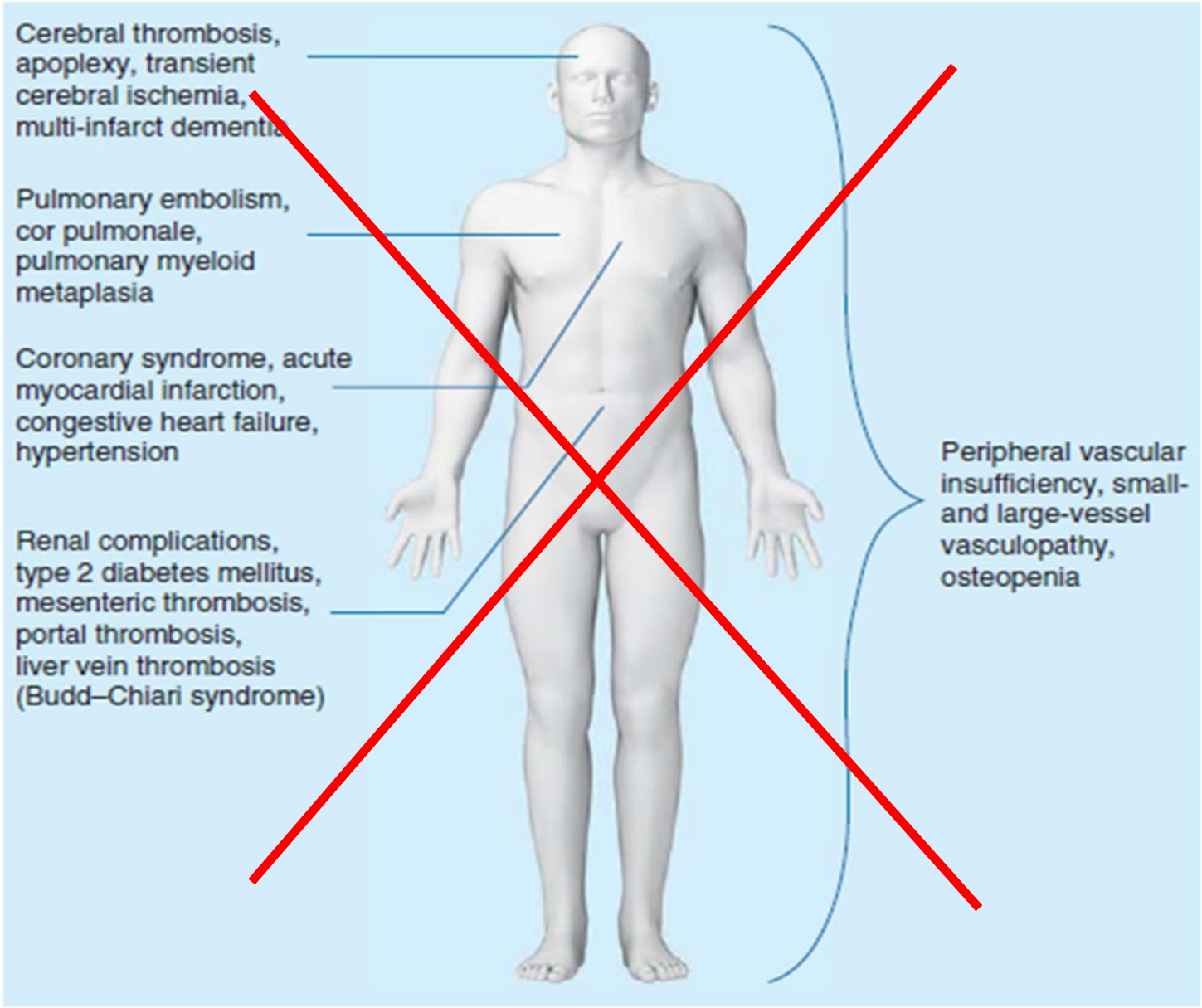


HGF: hepatocyte growth factor; IL: interleukin; TNF: tumour necrosis factor

Interferon Alpha2 in MPNs

- ✓ History of Interferons
- ✓ Biology and Mechanisms of Action
- ✓ The Biological Continuum (ET-PV-MF)
- ✓ The Novel Concept of Chronic Inflammation in MPNs
- ✓ Early Intervention Concept
- ✓ Minimal Residual Disease
- ✓ Interferon Resistance and Intolerability
- ✓ **Perspectives**

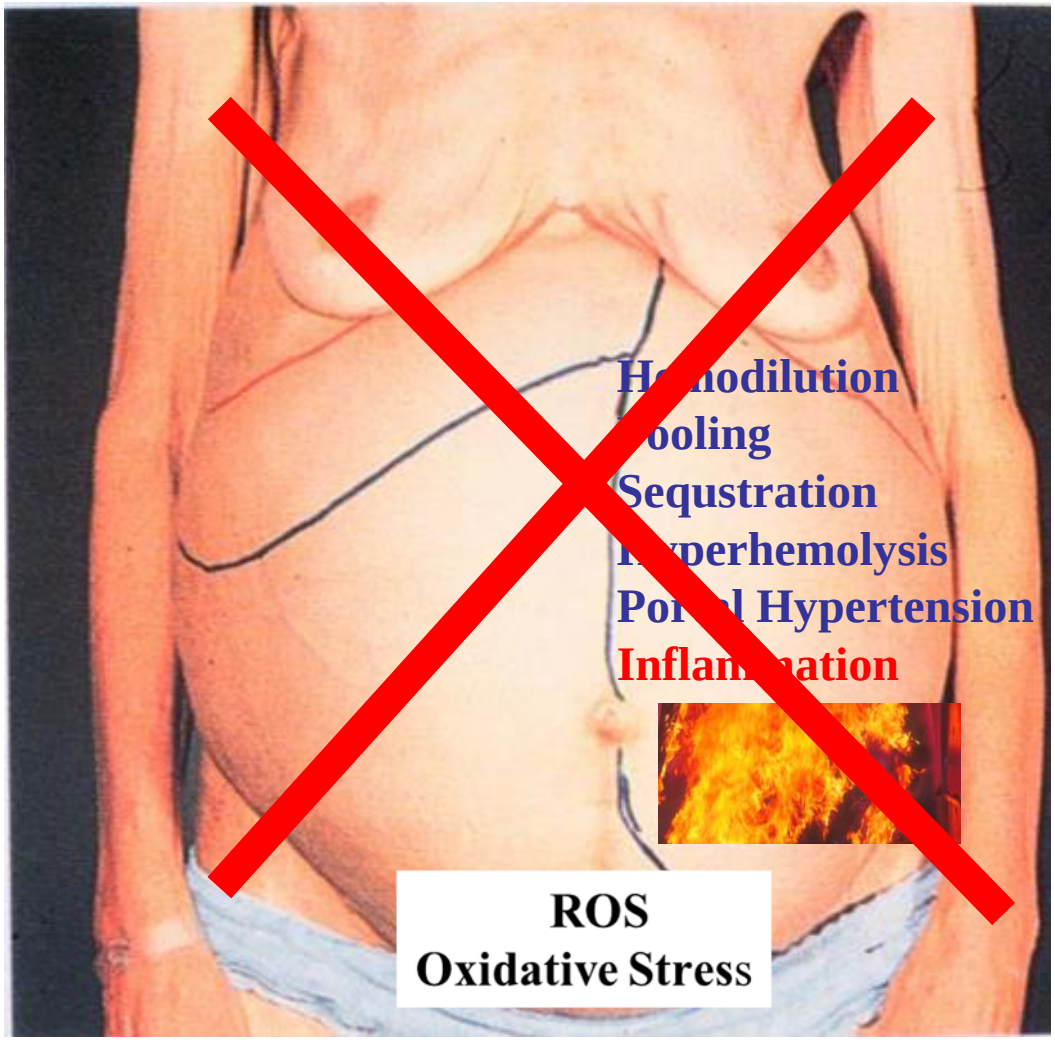
Chronic Inflammation Premature Atherosclerosis Cancer



The Inflamed Bone Marrow

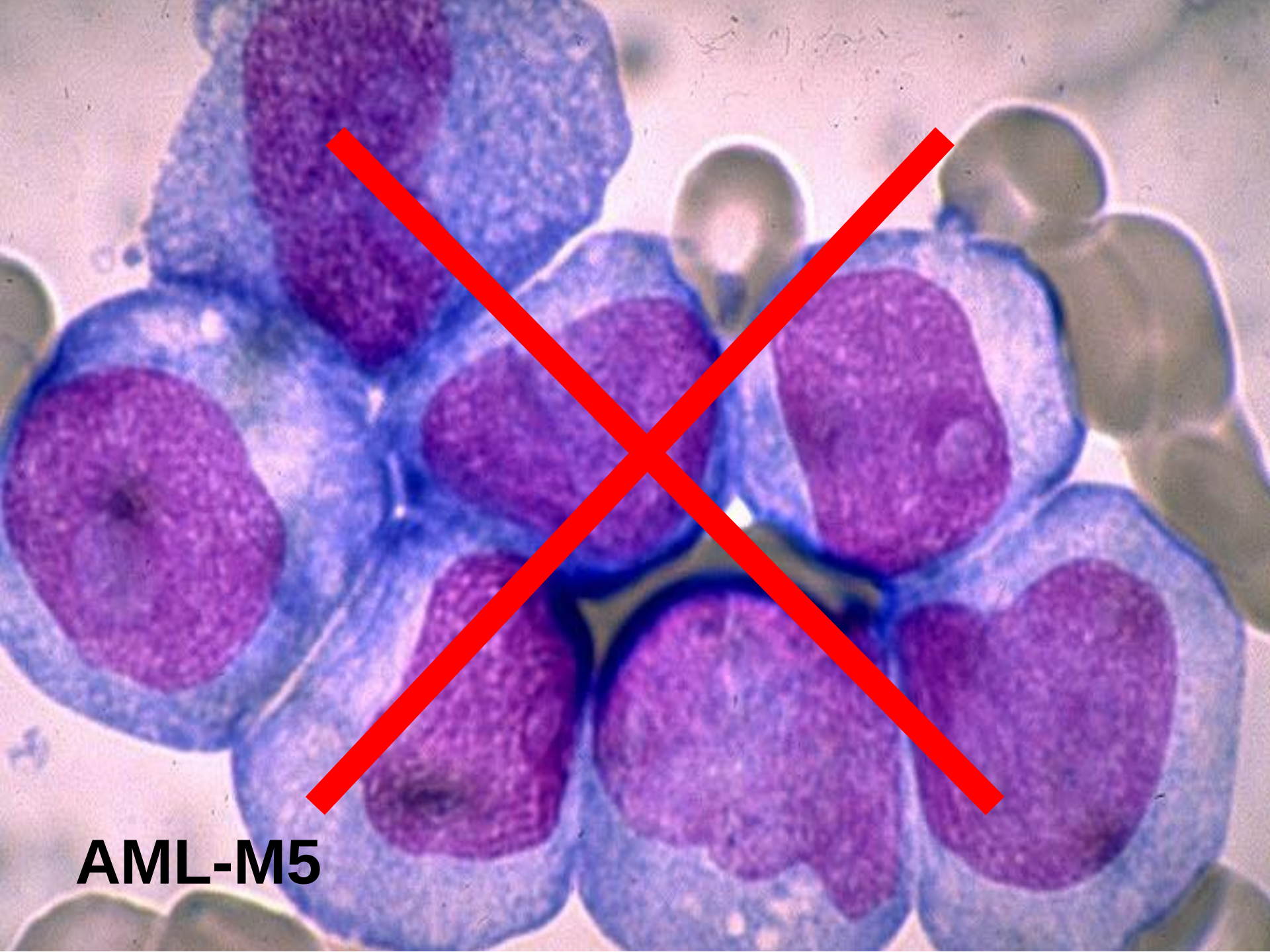
The chicks are flying prematurely (escaping) from the burning nest

ROS
Oxidative Stress

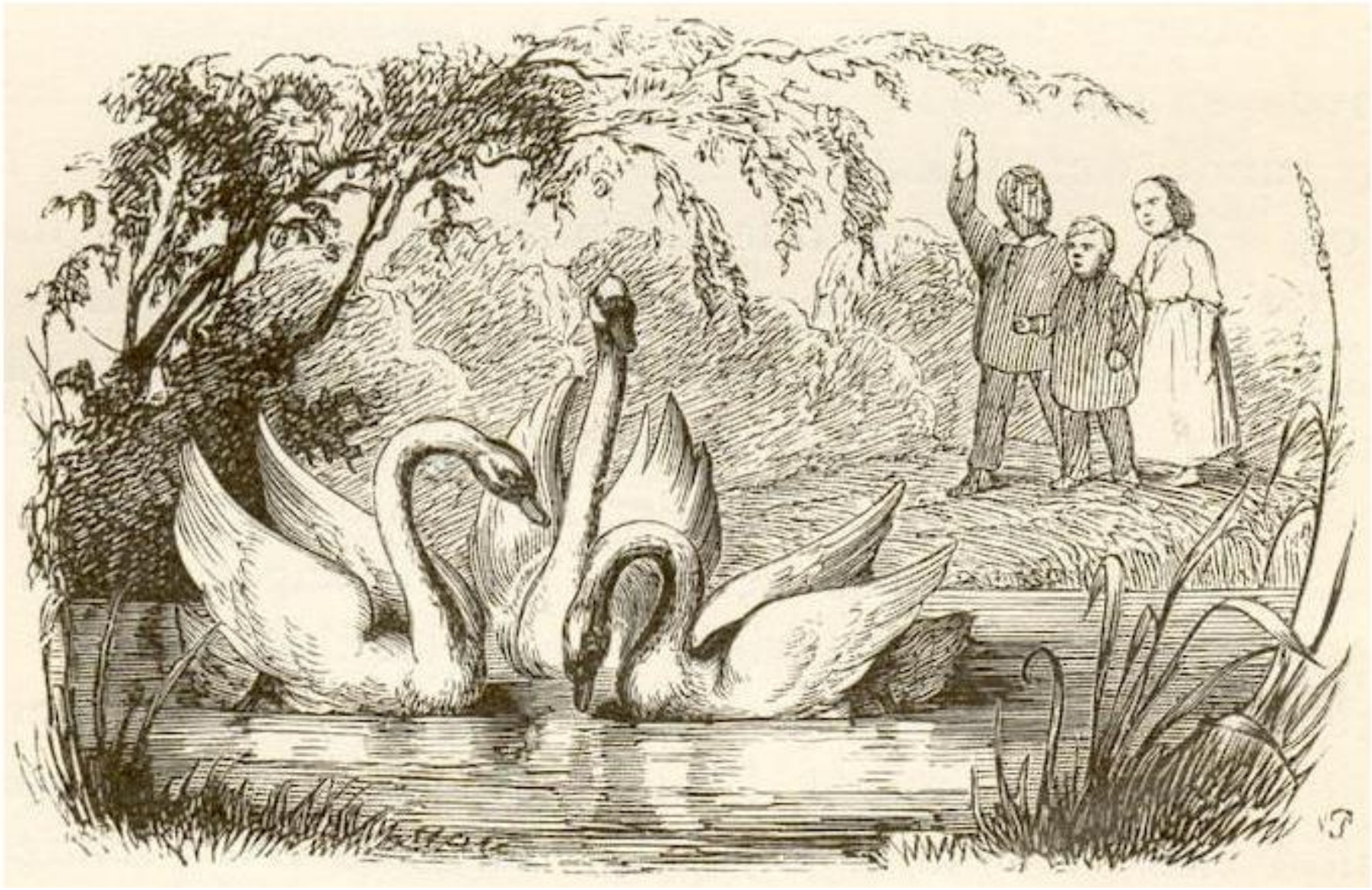


Myelofibrosis with huge splenomegaly

Anemia: bone marrow failure, hemodilution, pooling, sequestration, hyperhemolysis, portal hypertension, bleeding

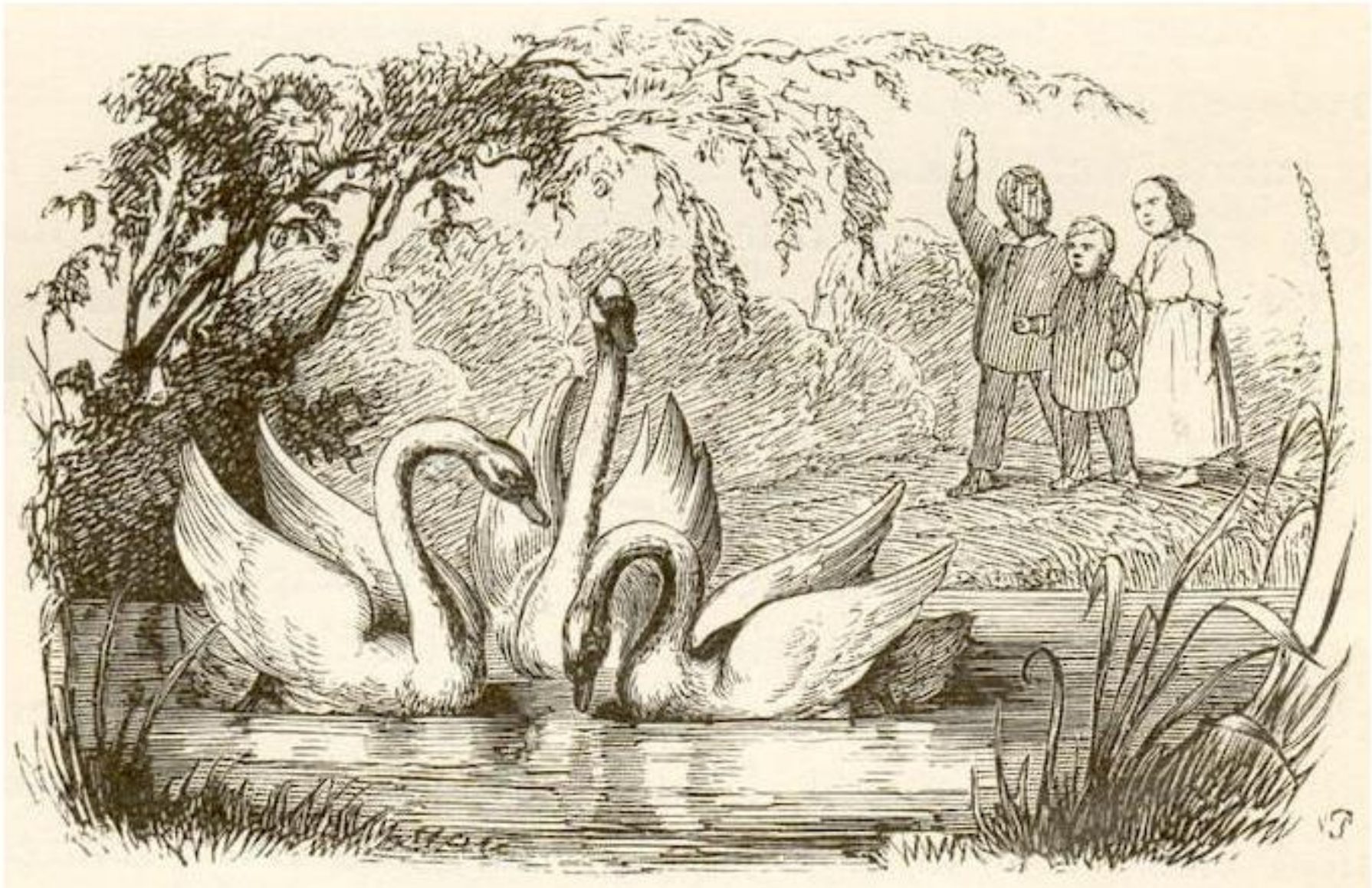

AML-M5

The Interferon Story
The Ugly Duckling Becoming The Beautiful Swan



The Ugly Duckling . A Fairy Tale by Hans Christian Andersen

Combination Therapy Interferon-alpha2 + Ruxolitinib
The Beautiful Swan Becoming Even More Beautful ?



The Ugly Duckling . A Fairy Tale by Hans Christian Andersen

Hans Christian Andersen

Photograph taken by Thora Hallager, 1869

Born	2 April 1805 Odense, Funen, Denmark
Died	4 August 1875 (aged 70) Copenhagen, Denmark
Occupation	Writer
Language	Danish
Nationality	Danish
Genre	Children's literature, travelogue
Signature	


The Future Looks Bright


Thank you for
your attention