Interferons in MPNs

Perspectives on The Early Interferon Concept

Combination Therapy with Ruxolitinib and Interferon

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Bologna
May 9, 2016
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

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
✓ 1986: FDA approval for treatment of HCL
✓ 1987: First report of efficacy in MF
✓ 1988: First report of efficacy in PV
✓ 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,†-3 Ruben A. Mesa,4 and Ronald Hoffman5

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

Table 3. Clinical trials of interferon in essential thrombocythemia

<table>
<thead>
<tr>
<th>First author, year</th>
<th>No. of patients</th>
<th>Response rate, %</th>
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<td>Giles, 1988</td>
<td>18</td>
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<tr>
<td>Bellucci, 1988</td>
<td>12</td>
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<td>Gugliotta, 1989</td>
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<td>26</td>
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<td>Giralt, 1991</td>
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<td>69</td>
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<td>Gisslinger, 1991</td>
<td>20</td>
<td>85</td>
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<td>Sacchi, 1991</td>
<td>35</td>
<td>85</td>
</tr>
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<td>Turri, 1991</td>
<td>10</td>
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<td>Kasparu, 1992</td>
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<td>Berte, 1996</td>
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<td>83</td>
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<td>Sacchi, 1998</td>
<td>11</td>
<td>100</td>
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<td>Radin, 2003</td>
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<td>Saba, 2005</td>
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<td>Langer, 2005</td>
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<td>75</td>
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<td>Samuelsson, 2006</td>
<td>21</td>
<td>70</td>
</tr>
<tr>
<td>Jabbour, 2007</td>
<td>13</td>
<td>70</td>
</tr>
<tr>
<td>Quintas-Cardama, 2009</td>
<td>39</td>
<td>81</td>
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Table 2. Clinical trials of interferon in polycythemia vera

<table>
<thead>
<tr>
<th>First author, year</th>
<th>No. of patients</th>
<th>Reduction of PHL, n (%)</th>
<th>Freedom from PHL, n (%)</th>
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<tr>
<td>Cacciola, 1991</td>
<td>11</td>
<td>9 (82)</td>
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<td>Cimino, 1993</td>
<td>13</td>
<td>10 (77)</td>
<td>4 (31)</td>
</tr>
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<td>Finelli, 1993</td>
<td>13</td>
<td>11 (85)</td>
<td>11 (85)</td>
</tr>
<tr>
<td>Turri, 1991</td>
<td>11</td>
<td>7 (64)</td>
<td>4 (36)</td>
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<tr>
<td>Papineschi, 1994</td>
<td>11</td>
<td>9 (82)</td>
<td>8 (73)</td>
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<td>Sacchi, 1994</td>
<td>22</td>
<td>21 (95)</td>
<td></td>
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<td>Muller, 1995</td>
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<td>7 (47)</td>
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<td>Taylor, 1996</td>
<td>17</td>
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<td>9 (53)</td>
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<td>Foa, 1998</td>
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<td>19 (50)</td>
<td>11 (29)</td>
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<tr>
<td>Gilbert, 1999</td>
<td>31</td>
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<td>NA</td>
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<td>Stasi, 1998</td>
<td>18</td>
<td>17 (94)</td>
<td>11 (61)</td>
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<tr>
<td>Hels, 1999</td>
<td>32</td>
<td>28 (87)</td>
<td>2 (6)</td>
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<tr>
<td>Radin, 2003</td>
<td>12</td>
<td>5 (42)</td>
<td>1 (8)</td>
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<tr>
<td>Silver, 2006</td>
<td>55</td>
<td>55 (100)</td>
<td>53 (96)</td>
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<tr>
<td>Samuelsson, 2006</td>
<td>21</td>
<td>7/9 (78)</td>
<td>4/9 (44)</td>
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<tr>
<td>Kiladjian, 2008</td>
<td>37</td>
<td>37 (100)</td>
<td>36 (97)</td>
</tr>
<tr>
<td>Quintas-Cardama, 2009</td>
<td>40</td>
<td>32 (80)</td>
<td>28 (70)</td>
</tr>
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Kiladjian JJ, Mesa RA, Hoffman R. Blood. 2011; 5;117(18):4706-15
The renaissance of interferon therapy for the treatment of myeloid malignancies

Jean-Jacques Kiladjian,1-3 Ruben A. Mesa,4 and Ronald Hoffman5

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

Table 4. Clinical trials of interferon in myelofibrosis

<table>
<thead>
<tr>
<th>First author, year</th>
<th>No. of patients</th>
<th>Response rate, %</th>
<th>Spleen size reduction, % of patients</th>
</tr>
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<tbody>
<tr>
<td>Hasselbalch, 1988</td>
<td>10</td>
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<td>10</td>
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<tr>
<td>Barosi, 1989</td>
<td>10</td>
<td>25</td>
<td>0</td>
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<tr>
<td>Gilbert, 1998</td>
<td>22</td>
<td>NA</td>
<td>58</td>
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<tr>
<td>Tefferi, 2001</td>
<td>11</td>
<td>0</td>
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<td>31</td>
<td>3</td>
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<td>Jabbour, 2007</td>
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<td>Iannotti, 2009</td>
<td>18</td>
<td>44</td>
<td>11</td>
</tr>
<tr>
<td>Silver, 2009</td>
<td>13</td>
<td>38</td>
<td>38</td>
</tr>
</tbody>
</table>

Kiladjian JJ, Mesa RA, Hoffman R. Blood. 2011; 5;117(18):4706-15
Interferon-Alpha2 Significantly Reduces The JAK2V617F-Allele Burden

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.

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
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
Semen Cell Wake Up Call

IFN-alpha - Myeloproliferative disorders - IFN-alpha

BCR-ABL → Chronic myeloid leukemia

JAK2 V617F → JAK2-positive thrombocytopenia

CALR → JAK2-positive polycythemia

Unknown mutation → JAK2-negative myeloproliferative disorder

Chronic phase → Accelerated phase → Blast crisis

Chronic phase → Accelerated phase

Leukemic transformation

myelofibrosis, cytopenias, increasing blasts, increasing white cells

Leukemic transformation
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

✓ 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
Chronic Inflammation ? → Chronic phase

Unknown genetic event

JAK2 V617F, CALR

Lympho-myeloid precursor Stem cell

Comorbidity Burden

ET  PV  Post PV MF  AML

100%

Chronic Inflammation ?

Chronic Inflammation ?

Chronic Inflammation ?
MPNs

ET – PV - PMF

A Human Inflammation Model ?

A Human Cancer Model ?

Chronic Inflammation – Genomic Instability - Clonal Evolution ?
Oxidative Stress – ROS-Genomic Instability - Cancer
A role for reactive oxygen species in $JAK2^{V617F}$ myeloproliferative neoplasm progression

C Marty$^{1,2,3}$, C Lacout$^{1,2,3}$, N Drouin$^{1,2,3}$, 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
**JAK2V617F** induces accumulation of ROS
**ROS** induces DNA-damage in stem cells
**DNA-damages induce genomic instability**
**Genomic instability induces mutations**

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

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ARTICLE INFO

Article history:
Received 1 July 2015
Received in revised form 29 August 2015
Accepted 4 September 2015
Available online xxx

Keywords:
Smoking
Chronic myelomonocytic stimulation
Essential thrombocytosis
Polycythemia vera
Primary myelofibrosis
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.

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A simplistic model of hematopoietic stem cell niches

An even more simplistic model of hematopoietic stem cell niches
The chicks are flying prematurely (escaping) from the burning nest.

Oxidative Stress – ROS Accumulation
Genomic Instability – Mutagenesis - Metastasis
Neutrophil Granules

MMM

- Mobilization
- Metastasis
- Myeloid Metaplasia

Oxidative Stress – ROS Accumulation
Genomic Instability – Mutagenesis - Metastasis
Transcriptional Profiling of Whole Blood Identifies a Unique 5-Gene Signature for Myelofibrosis and Imminent Myelofibrosis Transformation

Hans Carl Hasselbalch¹*, Vibe Skov², Thomas Stauffer Larsen³, Mads Thomassen², Caroline Hasselbalch Riley⁴, Morten K. Jensen⁴, Ole Weis Bjerrum⁵, Torben A. Kruse²

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 I, Herlev Hospital, University of Copenhagen, Herlev, Denmark, ⁵ Department of Hematology I, 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 thrombocytopenia 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.


Editor: Andre van Wijnen, University of Massachusetts Medical, United States of America

Received October 2, 2013; Accepted December 2, 2013; Published January 13, 2014

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Funding: The study has received grants from the The Danish Council for Independent Research | Medical Sciences (http://fwiu.dk/forskning-og-innovation/videnskabsforskning/udvalg/dff-sundhed-og-sygdom) The funders had no role in study design, data Collection and analysis, decision to publish, or preparation of the manuscript.

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

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A Unique Five Gene Signature in Myelofibrosis

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

ROS
Oxidative Stress

TNF-Alpha
IL-6, IL-8
IL-11, HGF
Inflammation in The Circulation

Circulating Leukocyte – Platelet Aggregates
Microcirculatory Disturbances
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 (e.g. 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?
Two Different Scenarios

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

Access IFN-alpha2
- ”Do no harm”
- Risk Stratification
- Normal blood counts
- Cytogenetic remission
- Molecular remission
- Normal bone marrow
- Minimal residual disease
Two Different Scenarios

No Access IFN-α2
- STOP HU

Access IFN-α2
- STOP IFN

Sustained

Complete HR
Molecular remission
Normal bone marrow
Minimal residual disease

A subset of patients
Two Different Scenarios

No Access IFN-alpha2

- HU (>10 yrs)
- Risk of
  - Skin cancer
  - MDS/AML
  - Second cancer

Access IFN-alpha2

- IFN-alpha2
- Risk of
  - Skin cancer
  - MDS/AML
  - 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?
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)
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

To cite this article: Cecilie Utke Rank, Ole Weis Bjerrum, Thomas Stauffer Larsen, Lasse Kjær, Karin de Stricker, Caroline Hasselbalch Riley & Hans Carl Hasselbalch (2015): 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, Leukemia & Lymphoma

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

- rp. IFN mar '99
- sep. IFN 1/3-08
- rp. IFN 13/8-13

* Bone marrow samples

**time, years**
Patient 9: Serial Measurements of JAK2V617F during and after discontinuation of interferon-alpha treatment

JAK2V617F allele burden, %

sep. IFN 23/9-10

time, years
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 Kőrmendiné Farkas,¹ Christian Fynbo Christiansen,¹ Hans Carl Hasselbalch,³ and Henrik Toft Sørensen¹

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

Patients with chronic myeloproliferative neoplasms, including essential thrombocytopenia (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)
Increased incidence of another cancer in myeloproliferative neoplasms patients at the time of diagnosis

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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

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Accepted for publication 1 July 2014
doi:10.1111/ejh.13410
Chronic Inflammation and Second Cancer in MPNs
Perspectives:

• Down-regulation of HLA-genes is a "tumor-escape mechanism" by which tumor cells escape the attack from potent immune cells e.g. cytotoxic 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

Interferon antibodies in thrombocythemia.

Merup M, Engman K, Paul C.

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.

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 ($< 400 \times 10^9/L$). 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.

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
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 Verstovsek1

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

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 JAK2V617F) 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 JAK2V617F and TET2 mutations at therapy onset had a higher JAK2V617F mutant allele burden and a less significant reduction in JAK2V617F allele burden compared with JAK2mutant/TET2 wild-type patients. These data demonstrate that pegylated interferon 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)

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

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.


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

Combination therapy with interferon and JAK1-2 inhibitor is feasible: Proof of concept with rapid reduction in JAK2V617F-allele burden in polycythemia vera

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Article info

Article history:
Received 13 March 2014
Received in revised form 13 May 2014
Accepted 19 May 2014
Available online 1 August 2014

Keywords:
Polycythemia vera
JAK2V617F-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 JAK2V617F 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 JAK2V617F-allele burden was reduced from 90% to 28%. Such a rapid decline in the JAK2V617F allele burden is highly unusual in PV-patients during low-dose IFN-alpha2 monotherapy and this finding warrants a prospective study with combination therapy.

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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 Mikkelsen¹, Lasse Kjær¹, Vibe Skov¹, Mads Emil Bjørn¹, Christen Lykkegaard Andersen², Ole Weis Bjerrum², Nana Brochmann¹, Daniel El Fassi³, Torben A Kruse⁴, Thomas Stauffer Larsen⁴, Torben Mourits-Andersen⁵, Claus Henrik Nielsen², Niels Pallisgaard¹, Mads Thomassen⁴ and Hans Carl Hasselbalch¹

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

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

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T-Cell

Enhanced Tumor Immune Surveillance

Killing of Tumor Cells

Interferon-Alpha

Statins

JAK2-Inhibitor
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
Statin therapy improves response to interferon alfa and ribavirin in chronic hepatitis C: A systematic review and meta-analysis

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ARTICLE INFO

Article history:
Received 6 December 2012
Revised 7 April 2013
Accepted 9 April 2013
Available online 16 April 2013

Keywords:
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

Cerebral thrombosis, apoplexy, transient cerebral ischemia, multi-infarct dementia

Pulmonary embolism, cor pulmonale, pulmonary myeloid metaplasia

Coronary syndrome, acute myocardial infarction, congestive heart failure, hypertension

Renal complications, type 2 diabetes mellitus, mesenteric thrombosis, portal thrombosis, liver vein thrombosis (Budd–Chiari syndrome)

Peripheral vascular insufficiency, small- and large-vessel vasculopathy, osteopenia
The chicks are flying prematurely (escaping) from the burning nest.
Myelofibrosis with huge splenomegaly
Anemia: bone marrow failure, hemodilution, pooling, sequestration, hyperhemolysis, portal hypertension, bleeding
The Interferon Story
The Ugly Duckling Becoming The Beautiful Swan

The Ugly Duckling . A Fairy Tale by Hans Christian Andersen
The Ugly Duckling. A Fairy Tale by Hans Christian Andersen

Combination Therapy Interferon-alpha2 + Ruxolitinib
The Beautiful Swan Becoming Even More Beautiful?
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The Future Looks Bright
Thank you for your attention