

UC Irvine

UC Irvine Previously Published Works

Title

The Ph-positive and Ph-negative myeloproliferative neoplasms: some topical pre-clinical and clinical issues

Permalink

<https://escholarship.org/uc/item/26j1294s>

Journal

Haematologica, 96(4)

ISSN

1592-8721

Authors

Van Etten, Richard A.
Koschmieder, Steffen
Delhommeau, Francois
et al.

Publication Date

2011-01-17

Peer reviewed

The Ph-positive and Ph-negative myeloproliferative neoplasms: some topical pre-clinical and clinical issues

Richard A. Van Etten,¹ Steffen Koschmieder,² Francois Delhommeau,³ Danilo Perrotti,⁴ Tessa Holyoake,⁵ Animesh Pardhanani,⁶ Ruben Mesa,⁷ Tony Green,⁸ Amr R. Ibrahim,⁹ Tariq Mughal,¹⁰ Robert Peter Gale,¹¹ and John Goldman¹²

¹Molecular Oncology Research Institute and Division of Hematology/Oncology, Tufts Medical Center, USA; ²Department of Medicine A (Hematology, Oncology and Pneumology), University Hospital, Münster, Germany; ³Laboratoire d'Hématologie et Immunologie, Hôpital Saint-Antoine, Paris, Université Pierre et Marie Curie, Paris and Inserm U1009, Institut Gustave Roussy, Villejuif, France; ⁴Dept. Mol. Virol. Imm. and Med. Genetics, Comprehensive Cancer Center, The Ohio State University, Columbus, USA; ⁵Paul O'Gorman Leukaemia Research Centre, University of Glasgow, UK; ⁶Mayo Clinic, Rochester MN, USA; ⁷Mayo Clinic, Scottsdale Arizona, USA; ⁸Department of Haematology, Addenbrookes Hospital, Cambridge UK; ⁹Department of Haematology, Imperial College London, UK; ¹⁰University of Colorado, Denver, CO, USA; ¹¹Celgene, Los Angeles CA, USA; and ¹²Department of Haematology, Imperial College London, UK

ABSTRACT

This review focuses on topical issues in the biology and treatment of the myeloproliferative neoplasms (MPNs). Studies in transgenic mice suggest that BCR-ABL1 reduces the fraction of self-renewing 'leukemic' stem cells in the bone marrow but that some of these cells survive treatment with imatinib. This also seems to operate in humans. Data from models also strongly support the notion that *JAK2*^{V617F} can initiate and sustain MPNs in mice; relevance to disease in humans is less clear. These data also support the hypothesis that level of *JAK2*^{V617F} expression influences the MPN phenotype: higher levels favor erythrocytosis whereas lower levels favor thrombocytosis. Although TET2-mutations were thought to precede *JAK2*^{V617F} in some persons with MPNs, it now appears that TET2 mutations may occur after *JAK2*^{V617F}. Further understanding of signal-transduction pathways activated in chronic myeloid leukemia suggests various possible targets for new therapies

including the WNT/beta catenin, notch and hedgehog pathways. Finally, the clinical role of the new JAK2- and BCR-ABL1-inhibitors is considered. Much further progress is likely in several of these areas soon.

Key words: myeloproliferative neoplasm, JAK2, Ph-negative, Ph-positive.

Citation: Van Etten RA, Koschmieder S, Delhommeau F, Perrotti D, Holyoake T, Pardhanani A, Mesa R, Green T, Ibrahim AR, Mughal T, Gale RP, and Goldman J. The Ph-positive and Ph-negative myeloproliferative neoplasms: some topical pre-clinical and clinical issues. *Haematologica* 2011;96(4):590-604. doi:10.3324/haematol.2010.035675

©2011 Ferrata Storti Foundation. This is an open-access paper.

Introduction

The advent of tyrosine kinase inhibitors (TKI) has proved to be an extraordinarily important advance in the management of patients with early phase Ph-positive chronic myeloid leukemia and has also influenced the direction of research into the underlying biology of leukemia and other tumors. The identification of the *JAK2*^{V617F} mutation in polycythemia vera and other Ph-negative myeloproliferative neoplasms (MPN) has made an extremely important contribution to our understanding of the basic biology of these disorders. There remain, however, many unanswered questions. This paper has three discrete but related themes. First, it reviews some of the murine systems that have been developed in recent years to model the *JAK2*^{V617F}-positive MPNs and BCR-ABL1-positive chronic myeloid leukemia (CML) with a view to defining those questions that might be answerable with appropriate model systems. Second, it reviews the very recent data rele-

vant to the issue of whether TET2 mutations predispose to development of an MPN or are 'merely' secondary events. Third, it summarizes briefly some of the recent results of using new agents to treat the Ph-negative MPN and describes some molecular pathways that could be exploited for therapy in the future.

(a) Transgenic models of JAK2-mutant MPNs

The MPNs and related conditions, many of which are characterized by dysregulated tyrosine kinase (TK) signaling,¹ are good candidates for mouse models. These models are typically established by expressing the relevant mutant signaling molecules, e.g. BCR-ABL1 in CML or mutant PTPN11 (in juvenile myelomonocytic leukemia) in mouse hematopoietic cells. There are two common strategies: (1) gene transfer into hematopoietic cells by retro- or lenti-viruses followed by transplantation; and (2) expression *via* a chromosomal transgene (for review see 2). Each method has advantages and dis-

Funding: RvE is supported in part by NIH grant HL089747. SK is supported in part by DFG grant KO2155/2-2. DP is supported in part by grants from the National Cancer Institute CA095512, the US Army and the CML Research Program, W81XWH-07-1-0270, and The Leukemia and Lymphoma Society. FD is supported by the MPD Foundation, the Association L. Fugain, the Fondation de France and the Ligue Nationale Contre le Cancer.

Manuscript received on October 15, 2010. Revised version arrived on December 14, 2010. Manuscript accepted on January 4, 2011.

Correspondence: Richard A van Etten, Molecular Oncology Research Institute and Division of Hematology/Oncology, Tufts Medical Center, 800 Washington Street, Box 5609, Boston, MA 02111, USA; Phone: +1.617.636.8110; E-mail: Rvanetten@tuftsmedicalcenter.org.

advantages for modeling and for pre-clinical evaluation of molecularly targeted therapies.

Several previous publications reported data on the retroviral strategy for modeling MPNs induced by JAK2^{V617F},³⁻⁶ JAK2 exon-12 mutants⁷ and MPL W515L/K mutants.⁸ However, the transgenic mouse field is rapidly catching up. Three new transgenic models of JAK2^{V617F}-induced MPNs were presented at the 2009 ASH meeting.⁹⁻¹¹ Here, we compare these new models with previously published models and consider what they can teach us about the pathophysiology of seemingly-corresponding MPNs (Table 1).

One of the major advantages of the transgene approach is the ability to express the mutant TK at near physiological levels. This contrasts with retroviral vector models in which relevant oncogenes are expressed at relatively high levels.⁵ This disparity might be particularly important for JAK2 as studies in cell lines indicate JAK2^{V617F} must associate with a type-I cytokine receptor (such as EpoR or MPL) for signaling activity.¹⁷ Consequently, competition between the retrovirus-encoded mutant JAK2 and endogenous, wild-type JAK2 may influence disease phenotype.¹⁸ The first published transgenic model from Skoda and co-workers¹² used a novel conditional inverted allele of human JAK2^{V617F} under the control of the human JAK2-promoter. A constitutively expressed Vav-Cre transgene or the interferon-inducible Mx-Cre transgene was used to flip the transgene into the correct orientation. Depending on whether Cre was expressed at high/sustained levels (Vav-Cre) or at lower/transient levels (Mx-Cre), recombination took place predominantly within or between tandemly repeated transgenes yielding different copy numbers and expression of mutant *versus* wild-type JAK2 with mRNA ratios of ~0.6 for Vav-Cre and ~1.0 for Mx-Cre. The Vav-Cre;FF (flip-flop) mice had normal hemoglobin and WBC levels but increased platelets. In contrast, the Mx-Cre;FF mice had variable but significant increases in WBCs and platelets and increased hemoglobin (170-210 g/L) and low plasma Epo levels. Skoda and co-workers concluded that lower expression of JAK2^{V617F} favoured an ET-like phenotype whereas higher expression favored a PV-like phenotype. Subsequently, reports of two transgenic JAK2^{V617F} models were published wherein mouse or human JAK2^{V617F} was expressed from an H-2Kb or Vav promoter.^{13,14} Here, there were significant differences in the phenotype and penetrance between founder mice (Table 1). However, in both models mice developed MPNs with variable degrees of polycythemia and thrombocytosis, extramedullary hematopoiesis, splenomegaly and Epo-independent erythroid colony (EEC) formation. Mice with lower relative levels of mutant JAK2 expression tended towards an ET-like phenotype with predominant thrombocytosis. These data support a correlation between level of JAK2^{V617F} expression and MPN phenotype.

Founder transgenic mice with constitutive expression of dysregulated TKs (such as BCR-ABL1 and JAK2^{V617F}) show marked phenotype variability. This may be related to different transgene insertion sites and/or to deleterious effects of transgene expression during embryogenesis,¹⁹ the consequence of which is to select for decreased transgene expression in survivors.

To circumvent these problems four recent JAK2^{V617F} models used "knock-in" approaches whereby the mutation was introduced into the normal JAK2 locus, so that

the mutant JAK2 would be expressed physiologically. In two models, mutant JAK2 expression was further conditionally activated or regulated by Cre-lox recombination (Table 1). Mice in all four models developed MPN phenotypes. In the model from Golam Mohi and colleagues,^{9,15} a mouse JAK2^{V617F} allele was expressed after Mx-Cre-mediated recombination. Heterozygous and homozygous transgenic mice developed an MPN with polycythemia and thrombocytosis with a more marked phenotype in homozygotes. In the model described by Tony Green and co-workers,¹⁰ mice expressing a conditional human JAK2^{V617F} allele developed predominantly an ET-like phenotype with thrombocytosis and moderate polycythemia but not splenomegaly or myelofibrosis. The model reported by Jean-Luc Villeval and colleagues expressed a knock-in mouse JAK2^{V617F} allele constitutively;¹¹ these mice developed a severe MPN phenotype with polycythemia, thrombocytosis, splenomegaly and myelofibrosis.

Most recently, Ebert and colleagues reported the phenotype of a similar constitutively expressed murine Jak2^{V617F} knock-in allele, where mice developed fatal MPN with polycythemia and splenomegaly but lacking thrombocytosis and myelofibrosis.¹⁶ The MPN was transferred to secondary recipients by transplantation of stem (Lin⁻/Sca⁺/Kit⁺, LSK) cells, but not by committed progenitors.

What can we learn from these diverse mouse models of aberrant JAK2-expression? First, although considerable data suggest that one or more mutation(s) may antedate the JAK2^{V617F} mutation in persons with MPNs,²⁰ the high prevalence of one or more MPN phenotypes in the knock-in transgenic models coupled with the polyclonal MPN observed in the retroviral models⁵ strongly suggests that JAK2^{V617F} can initiate and sustain MPN in mice. Whether this conclusion applies to humans is unknown.

Second, one of the most interesting questions is how one genetic lesion, JAK2^{V617F}, can cause diverse MPN phenotypes. Is dose the answer? For example, in humans homozygosity for JAK2^{V617F} occurs exclusively in PV, and not in ET.²¹ In these models, there is support for the concept that expression of JAK2^{V617F} at levels similar to or higher than endogenous JAK2 is associated with erythrocytosis whereas lower expression levels favor thrombocytosis. In the most recent and more "physiological" knock-in models, JAK2^{V617F} expression is at levels equal to endogenous JAK2. In the models of Green and Villeval mice developed polycythemia, albeit to different extents.

However, the model reported by Mohi does not fit this paradigm: JAK2^{V617F} expression was (unexpectedly) only about half that of JAK2 allele yet mice developed polycythemia. Homozygosity increased platelets further (Table 1).

There are various possible reasons for these phenotype differences including divergence between human and mouse JAK2 and variability in mouse strains (which influences the MPN phenotype in retroviral models)⁵. Lastly, we should recall that these mice differ from humans with MPNs in that the mice lack a normal population of hematopoietic stem cells and are wholly dependent on JAK2^{V617F}-associated hematopoiesis for blood cell production. As always, we can learn much from mouse models but they rarely replicate precisely the disease in humans. In fact, the diversity of MPNs in humans with a seemingly canonical JAK2 mutation far exceeds the aforementioned diversity of mouse models. It is well known that

familial MPNs with JAK2 mutation have diverse phenotypes.²² This must mean factors other than JAK2 operate.

(b) Mouse models of Ph-positive MPNs

Mouse models of Ph-positive CML are critical for the understanding of functionally relevant cellular and molecular abnormalities and were developed using three approaches: (1) xenotransplants; (2) retroviral transplantation models; and (3) transgenic mice. Although xenotransplants of human CML cells to mice allow us to study and manipulate human cells *in vivo*, engraftment of human cells in NOD/SCID or similar mouse strains is generally less efficient than transplants using allogeneic or syngeneic murine cells. Moreover, although transplantation of human CML acute phase cells causes acute leukemia in NOD/SCID mice,²³ chronic phase (CP) cells do not cause disease despite obvious engraftment.^{23,24} In contrast, retroviral expression of BCR-ABL1 in hematopoietic stem and progenitor cells results in versatile mouse models of CML facilitating functional analysis of BCR-ABL1 mutants, identification of critical target genes and cooperating oncogenes and assessment of new therapies.²⁵⁻²⁸ This topic was reviewed by van Etten.²⁹ In addition, transgenic mouse models were generated that express BCR-ABL1 under the control of different promoters and enhancers. These models shed light on the nature of the CML-initiating cell and CML stem cell biology. Here we update only the transgenic mouse models (P210 BCR-ABL1).

Honda and co-workers crossed Tec promoter-driven BCR-ABL1 transgenic mice with BXH2 mice in a screen for common integration sites and identified a novel cooperating gene, Zfp423.

Coordinate overexpression of BCR-ABL1 and Zfp423 induced acute myeloid and lymphoid (B and T cell) leukemias in mice reminiscent of acute phase.³⁰ These investigators reported a similar outcome by crossing Tec-BCR-ABL1 transgenic mice with Bcl11^{+/-} or H2AX^{+/-} mice.³¹

Sánchez-García and co-workers generated transgenic mice expressing BCR-ABL1 under the control of a Sca-1 promoter³² to recapitulate CML as a stem cell disease in mice. Interestingly, embryonic lethality, commonly described previously in BCR-ABL1 transgenic mice,¹⁹ was not reported.³² Within 6-18 months, the transgenic mice developed a CML-like disease characterized by mildly increased WBC, increased neutrophils, splenomegaly and hepatomegaly and ultimately acute leukemia resembling the acute phase of CML in humans. The authors reported that cross-breeding of two founder lines shortened development of this phenotype to 1-2 months. BCR-ABL1 expression was confined to the Sca-1⁺ cell population including hematopoietic stem cells (HSCs). However, the fact that a significant proportion of mice developed solid cancers (10% lung adenocarcinoma, 3% liver cancer, 2% gastrointestinal stromal cancers and others) shows that Sca-1 driven oncogene expression in non-hematopoietic cells may hamper some aspects of transgenic mouse modeling.

Koschmieder and co-workers generated BCR-ABL1 expressing mouse strains using the murine stem cell leukemia gene (SCL) 3' enhancer.³³ After inter-crossing SCLtTA and TRE-BCR-ABL1 mice to generate double-transgenic SCLtTA/BCR-ABL1 mice, BCR-ABL1 expression was induced by tetracycline withdrawal. These mice developed a CML-like disease within 30 to 120 days (increased WBC and neutrophils, splenomegaly and granulocyte infiltration of the liver, gut and lung); these fea-

tures were reversible and re-inducible. These data suggest that developing a CML phenotype requires sustained BCR-ABL1 expression. Furthermore, Schemionek and co-workers from the same group showed that this CP-CML is transplantable into congenic mice.³⁴ The LSK population, but not the Lin/Sca1/Kit⁺ progenitor or the granulocyte compartments, contains the CML-initiating cell. BCR-ABL1 reduces long-term repopulating (LT-) HSC numbers in the bone marrow and induces their differentiation. Spleen LT-HSC numbers are not reduced. Further experiments showed that the leukemia-inducing potential of BCR-ABL1 positive bone marrow is compromised by serial transplantation. These data suggest that BCR-ABL1 decreases the fraction of self-renewing stem cells in the bone marrow. Interestingly, this is very similar to a recently developed knock-in mouse model for JAK2^{V617F} which showed reduced leukemia stem cell (LSK cell) numbers and impaired repopulation potential in the JAK2^{V617F}-positive mice.³⁵ This parallel reinforces important similarities of the JAK2^{V617F} and BCR-ABL1 oncogenes *in vivo*. The CML-like disease in transplant recipients is reversible by stopping BCR-ABL1-expression and by giving imatinib.

However, CML-initiating cells survive independently of BCR-ABL1 expression and give rise to recurrent CML when the BCR-ABL1 is re-expressed or imatinib is discontinued.

Zhang and colleagues³⁶ were able to confirm these results. Using quantitative competitive repopulation assays they showed that the frequency of functional HSCs was reduced substantially in BCR-ABL1-positive mice compared to controls. Moreover, although one in 6 Flt3⁺CD150⁺CD48⁻ LSK cells possessed repopulating activity, only one in 80 cells caused leukemia in transplanted mice. This may reflect the presence of BCR-ABL1-negative stem cells and thus allows the study of BCR-ABL1- positive- and -negative hematopoiesis in the same mouse.

This group provided evidence for significant *in vivo* activity of the combination of imatinib and the nuclear histone deacetylase (HDAC) inhibitors LAQ824 or LBH589.³⁷ BCR-ABL1-transgenic mice treated with imatinib and LBH589 had prolonged leukemia-free survival after discontinuing treatment compared with mice treated with either drug alone. Expression of one of the putative targets, MCL-1, was strongly inhibited by combination therapy, suggesting a role for MCL-1-inhibition in controlling or killing CML stem cells.

Transgenic mice may be valuable to identify molecular target that may prove clinically useful. For example, Perrotti and co-workers showed that RNA binding proteins hnRNA-A1, -K, and -E2 are increased in BCR-ABL1 positive transgenic mice and that this results in SET activation and suppression of protein phosphatase 2A (PP2A).^{38,39} These authors reported that the PP2A activator, FTY-720, induces apoptosis of BCR-ABL1 positive LSK cells in SCLtTA/BCR-ABL1 mice.³⁹ This work is described in greater detail later in this paper.

Finally, Sengupta and co-workers showed that BCR-ABL1-positive spleen cells can transplant CML-like disease. These cells have decreased adhesion and increased migration compared to BCR-ABL1 negative cells.⁴⁰ By crossing BCR-ABL1 transgenic mice with Rac2^{-/-} mice these investigators showed that Rac2 is critical for BCR-ABL1-induced disease and for the proliferation and survival of leukemia stem cells (LSC).⁴⁰

Together with retroviral transplantation and as xeno-

Table 1. Comparison of transgenic *JAK2*^{V617F} mouse models of MPN.

Lab	JAK2 source/ Expression	Strategy	Phenotype and Comments	Ref.
Skoda	Human/ Conditional Tg	Human <i>JAK2</i> BAC Tg containing 5' promoter and exons 1-12, with <i>JAK2</i> ^{V617F} mutation in an inverted cDNA (exons 13-25) flanked by loxP sites. Recombination by Vav-Cre (sustained/high level Cre) or Mx-Cre + plpC (transient/moderate Cre). Vav-Cre flipped ~80% of cDNAs into sense orientation with Tg copy # of ~2.0, while Mx-Cre flipped ~70% with ~5.7 Tg copies.	By QRT-PCR using species-specific primers, Tg: endogenous <i>JAK2</i> was ~0.6 (Vav-Cre) and 1.0 (Mx-Cre). At 20 wks, Vav-Cre;FF (flip-flop) mice had normal Hgb and leukocytes but predominant thrombocytosis (plts 2000-4000 x 10 ⁹ /L), while at 20 wks post-plpC, Mx-Cre;FF mice had variable but significant increases in WBC and plts, accompanied by an increased Hgb (17-21 g/dL) and suppressed Epo levels. Both strains developed EMH, splenomegaly, and MF, but neither had EEC. Conclusion: lower expression of <i>JAK2</i> ^{V617F} favors an ET-like phenotype, while higher levels favors a PV-like phenotype with expansion of erythroid cell mass. MPN phenotype from Mx-Cre;FF transferred by BMT. (Two founders with a non-floxed BAC Tg died before breeding, while a second conditional FF Tg founder had no phenotype upon Mx-Cre induction.)	(12)
Shimoda	Mouse/ Constitutive Tg	Mouse <i>Jak2</i> ^{V617F} cDNA Tg driven by the H-2Kb promoter. Two founders (lines 1 and 2), both born at expected Mendelian ratio. Background: mixed B6;DBA→B6.	By QRT-PCR, line 1 expressed 0.45x as much mutant <i>Jak2</i> as endogenous (diploid) wt <i>Jak2</i> , and line 2 expressed ~1.35x as much as endogenous. Line 1 (incomplete penetrance): 19% had modest polycythemia (Hgb 18-20g/dL), 35% had thrombocytosis (1400-3000 x 10 ⁹ /L), and a correlation between higher Tg expression and PV-like phenotype. Line 2: uniform and extreme neutrophilia (WBC 100-400 x 10 ⁹ /L) and thrombocytosis (2000-5000 x 10 ⁹ /L) from one month, with anemia (Hgb 9-10 g/dL) unaccompanied by MF. Leukocytosis and thrombocytosis declined w/time while anemia persisted, coincident w/increased MF. Both lines had EMH, splenomegaly, and EEC.	(13)
Zhao	Human/ Constitutive Tg	Human <i>JAK2</i> ^{V617F} cDNA Tg driven by the Vav promoter. Three founders generated, two Tg lines maintained (A and B). Background: mixed B6;DBA→B6, F0-F6. Tg copy number ~13 for A and ~2 for B, single chromosome integration site for each.	By QRT-PCR with species-specific primers, mutant <i>JAK2</i> transcript level was ≤ 1/16 th that of endogenous/wt murine <i>Jak2</i> , even for line A. Line A developed prominent thrombocytosis (plts 1200-5000 x 10 ⁹ /L) and modest leukocytosis and erythrocytosis (mean Hgb 18.1), with EMH, splenomegaly, EEC, and moderate MF. Line B had a much milder phenotype, with modest thrombocytosis (mean 1300 x 10 ⁹ /L) and splenomegaly and no EEC or MF.	(14)
Mohi	Mouse/ Conditional KI	Targeting vector with a loxP-flanked cassette containing mouse <i>Jak2</i> cDNA (exons 13-24)-polyA-transcription stop, followed by genomic <i>Jak2</i> exons 15-17 (with V617F mutation in e15), was knocked into the <i>Jak2</i> locus between exons 12 and 17. Background: mixed B6;129Sv.	Hetero- or homozygous Mx-Cre;KI mice were induced w/plpC. By direct sequencing of QRT-PCR products (T:G ratio), mutant allele expressed at ~0.53x the level of wt <i>Jak2</i> . Phenotype at 12 wks post-plpC was 100% penetrant; both heterozygous and homozygous Mx-Cre; <i>Jak2</i> ^{V617F} mice had polycythemia (mean Hgb ~22 and ~18 g/dL, respectively), but leukocytosis (mean ~28 and ~57 x 10 ⁹ /L) and thrombocytosis (mean 1700 and 3500 x 10 ⁹ /L) were more marked in homozygotes, as was splenomegaly, EEC frequency, and degree of MF. Polycythemia phenotype was transplantable by BMT.	(15)
Green	Human/ Conditional KI	Knocked in a human <i>JAK2</i> ^{V617F} cDNA Tg, preceded by a floxed pGK neo pA cassette, into the murine <i>Jak2</i> exon 2 (containing AUG) locus. Background: mixed B6;129S7/SvEvBrd.	6 week-old Mx-Cre; <i>JAK2</i> ^{V617F} /+ mice were induced with plpC. Species-specific QRT-PCR for human and mouse <i>Jak2</i> found equivalent expression. plpC-treated mice developed a chronic MPN characterized by moderately increased hematocrit (~18 g/dL vs. 16 control) and platelet counts (1700 x 10 ⁹ /L vs. 1300 control), and EECs, but no splenomegaly or MF. Plasma Epo levels normal. MPN phenotype transferred by BMT.	(10,35)
Villeva	Mouse/ Constitutive KI	Knocked in a mouse <i>Jak2</i> exon13 containing the V617F mutation into the <i>Jak2</i> locus, removed the FRT-flanked neo cassette by crossing to FLP transgenic mice. Background: mixed B6;129Sv.	Mice developed MPN at 5 months of age, characterized by marked polycythemia (Hct 71%±3.6%), leukocytosis (WBC 79±11 x 10 ⁹ /L) and thrombocytosis (4400±700 x 10 ⁹ /L). By allele-specific QRT-PCR, there were equal amounts of wild-type and mutant <i>Jak2</i> mRNA expressed. Mice also exhibited splenomegaly, EMH, EECs, and age-related MF. MPN phenotype was transferred to recipients by BMT.	(11)
Ebert	Mouse/ Conditional KI	Knocked in an inverted mouse <i>Jak2</i> exon 13 containing the V617F mutation into the <i>Jak2</i> locus and recombined and inverted the exon in the germline. The parental allele was null and could not be homozygosed. Background: B6 backcrossed.	Mice developed fatal MPN by 8 weeks of age that was 100% penetrant and fatal with median survival of 146 days, characterized by polycythemia and splenomegaly but without thrombocytosis or EEC. Level of expression of mutant <i>Jak2</i> allele not determined. MPN phenotype transferred by transplantation of stem (LSK) cells.	(16)

BAC, bacterial artificial chromosome; BMT, bone marrow transplantation; EEC, endogenous (Epo-independent) erythroid colonies; EMH, extramedullary hematopoiesis; KI, knock-in; MF, myelofibrosis; QRT-PCR, quantitative real-time polymerase chain reaction; Tg, transgene

transplant models, transgenic mouse models can be exploited in studies of the CML phenotype, of CML stem cell biology, and of new therapeutic strategies.

(c) Role of TET2 in the Ph-negative MPNs

Why is it difficult to identify pre-JAK2^{V617F} events in MPN? It is quite likely that even if these events are important they have subtle or no clinical consequence by themselves. Shortly after the JAK2^{V617F} mutation was described studies of clonality of hematopoietic cells suggested the existence of pre-JAK2^{V617F} mutations, at least in some people.⁴¹⁻⁴³

The first pre-JAK2^{V617F} event described was deletion of the long arm of chromosome 20 (del[20q]). In some patients with MPNs del(20q) seemed to precede the JAK2^{V617F} mutation. In others this sequence was reversed.^{20,44} Thus del(20q) appeared independent and was unlikely to predispose to JAK2^{V617F} mutation. Other data from persons with MPNs and JAK2^{V617F} mutation whose disease transformed to blastic phase (BP) gave an intriguing picture: leukemia cells from one-half of the cases were JAK2^{V617F}-negative.^{4,43,45} These data argue for the existence of molecular lesions that might predispose to JAK2^{V617F} mutations in MPN and/or to other JAK2^{V617F} independent acute myeloid leukemias.

In 2009, mutations in TET2 and ASXL1 were identified in various myeloid neoplasms, including in around 10% of patients with MPNs.⁴⁶⁻⁴⁹ TET2, like other members of the TET family enzymes, presumably has an important function in DNA demethylation. It has recently been shown that the murine Tet proteins catalyze the conversion of 5 methylcytosine to 5 hydroxymethylcytosine on DNA.⁵⁰ Thus TET2 mutations are thought to alter hematopoietic stem cell (HSC) functions and myeloid development *via* epigenetic modifications. These mutations were ideal pre-JAK2^{V617F} candidate events. Indeed, studies of clonality in

appropriate patients with typical MPNs showed that most cells had both mutations but that some cells had a TET2 mutation without a JAK2 mutation^{44,47} suggesting that the TET2 mutation was antecedent. In one case, the TET2 mutant clone acquired JAK2 and MPL mutations in distinct sub-clones.⁵¹ Is this the whole story? Unfortunately, no. Data from other patients with MPNs show a JAK2 mutation preceding the TET2 mutation.^{44,52,53} Other patients with MPNs had clones with either a JAK2 or TET2 mutation but not both.⁴⁴ Similar studies of ASXL1 mutations have not been published but the same complexity is expected. What if people with these mutations develop transformation to blastic phase? In some instances, blast phase cells with TET2 defects had no TET2 mutation detected before transformation.^{51,54}

In contrast, in 2 of 3 cases of MPN with ASXL1 mutations after transformation the mutation was also detected before transformation.^{51,54} This seeming discordance with TET2 should be tempered as TET2 mutations were detected in 10-20% of *de novo* cases of acute myelogenous leukemia (AML) and MPNs undergoing transformation to blastic phase.⁵⁵

It is, therefore, possible that TET2 mutations, but also ASXL1 and del(20q), have different roles in initiating MPNs and causing AML alone or are associated with other molecular abnormalities.^{51,54}

What of familial MPNs? The first studies of TET2 mutations in familial cases reported no inherited mutations.⁵² However, a recent report described sisters with a germline mutation of TET2.⁴⁴ One presented with a JAK2^{V617F}-positive PV; the second was normal. This is the first evidence that TET2 mutations can be present in hematopoietic cells with no clinical consequence for decades. These data suggest no or only a very subtle effect on hematopoiesis of TET2 mutations. It could give a slight proliferative advan-

Table 2. Current trials of targeted therapies for Ph-negative MPNs.

Drug	Target	Phase	Disease	Efficacy	Toxicity	Ref
JAK2 Inhibitors						
INCB18424	JAK2 JAK1	III	MF PV/ET	Splenomegaly Symptoms	Anemia Thrombocytopenia	(114;115)
TG101348	JAK2 FLT3	II	MF	Splenomegaly Symptoms	Anemia Thrombocytopenia Gastrointestinal	(116)
SB1518	JAK2 FLT3	II	MF	Splenomegaly Symptoms	Gastrointestinal	(117)
CEP701	JAK2 FLT3	II	MF PV/ET	Splenomegaly Symptoms	Gastrointestinal Anemia Thrombocytopenia	(118;119)
Other Targets						
LBH-589	HDAC	II	MF	Splenomegaly Anemia	Anemia Thrombocytopenia Gastrointestinal	(120)
RAD-001	mTOR	II	MF	Splenomegaly Symptoms	Minimal	(121)
Pomalidomide	IMiD	III	MF	Anemia	Minimal	(122)
Pegylated Interferon Alpha-2a	Biological	III	PV/ET	Erythrocytosis Thrombocytosis Symptoms	Myelosuppression Depression	(123;124)

tage to HSCs by causing a few “extra” mitoses, which could prepare the way for subsequent oncogenic mutations. This is reminiscent of the “fertile ground” hypothesis proposed for the JAK2 46/1 haplotype to explain the predisposition to acquire a JAK2^{V617F} MPN.⁵⁶⁻⁶⁰ Intriguingly, this haplotype is the only marker that we can reasonably call a “pre-JAK2^{V617F} condition” to date, although most individuals who carry it will never develop any JAK2^{V617F} MPN.

(d) What molecular changes in the CML stem cells are critical for leukemia initiation or progression?

First and second generation TKI given to chronic phase (CP) CML patients substantially reduce the number of leukemia cells in the blood and bone marrow with improved survival. However, the story of the rationally-designed TKIs (imatinib, nilotinib, dasatinib and bosutinib) seems to have reached an ‘impasse’.⁶¹ For example, TKI-based therapies are largely ineffective in blast phase (BP) CML. More importantly, they are apparently unable to eradicate CML leukemia stem cells (LSCs). This raises several important questions; (1) Is BCR-ABL1 expression and/or activity essential to maintain the LSC reservoir in CP and BP? (2) Does BP result from genetic or epigenetic events that occur in LSCs or in more committed cells like granulocyte-macrophage progenitor (GMP) cells; and (3) what role, if any, does BCR-ABL1 play in blastic transformation?

Molecular events critical for the CML LSC

Considerable data indicate that TKI do not kill quiescent Ph-positive LSCs (defined as lineage-negative CD34⁺/CD38⁻/CD90⁺ cells capable of self-renewal and of inducing transplantable leukemia in mice). Consequently, there is agreement that BCR-ABL1 expression and/or activity is not essential for self-renewal and survival of the CML LSC.⁶²

Several observations in this context are of interest: (1) increased genomic instability correlating with levels of reactive oxygen species (ROS) in quiescent and proliferating CML LSCs and progenitors is associated with BCR-ABL1 expression;⁶³ (2) enhanced activation of the β -catenin and hedgehog pathways^{64,65} is important for CML LSC survival and self-renewal;⁶⁶⁻⁷⁰ (3) treatment with the PP2A activator FTY720 decreases the number of Ph-positive but not of normal quiescent HSCs;^{39,65} (4) activity of FoxO transcription factors also appears important for maintaining CML LSC quiescence;⁷¹ (5) ALOX5-regulated MSR1 gene might be critical for maintaining CML LSCs; (6) BMI-1 expression decreases bone marrow homing and increases proliferation and maturation of leukemia Lin⁻/Sca⁺/Kit⁺ HSCs;⁷² (7) deficient RAC2 expression decreases survival of CML LSCs;⁷³ (8) loss of BCL6 expression results in decreased self-renewal of BCR-ABL1-transduced bone marrow cells;⁷⁴ and (9) oxygen levels in the bone marrow microenvironment may also be responsible for the quiescence and TKI-resistance of CML LSCs.^{75,76} These diverse data suggest that CML LSC behavior is controlled by different factors. The various pieces of this puzzle need to be molecularly and functionally inter-connected and could then form the basis for new therapeutic interventions.

Molecular events critical for the CML blastic phase granulocyte/macrophage progenitor

Lymphoblastic transformation may be characterized by non-random genetic aberrations (involving CDKN2A/B

Table 3. Potential strategies to target CML LSC.

LSC apoptosis	Microenvironment/self-renewal/ quiescence/ differentiation	Immune therapy
BMS-214662 ^{125,126}	CXCR4 inhibitors	RISCT (127;128)
HDAC inhibitors ³⁷	Hedgehog pathway inhibitors ^{65,67}	Vaccines ¹²⁹⁻¹³²
Proteasome inhibitors ¹³³⁻¹³⁵	WNT pathway inhibition	
Autophagy inhibitors ¹³⁶	Cytokines e.g. G-CSF ¹³⁷⁻¹³⁹	

and IKZF1) and by expression of the activation induced deaminase (AID) mutator enzyme.⁷⁷⁻⁷⁹ In contrast, myeloid blastic transformation seems correlated with increased BCR-ABL1 expression and kinase activity in CD34⁺ BP progenitors.⁶¹ BCR-ABL1 dose and kinase-dependent loss of PP2A activity and ROS induced genomic instability appear critical for the epigenetic and genetic heterogeneity characteristic of myeloid transformation.^{63,80} Increased BCR-ABL1 activity also appears essential for the differentiation-arrested phenotype of granulocyte/macrophage progenitors (GMP) in BP through alteration of the hnRNP-E2-CEBP α -miR-328 network.⁸¹ There is a concomitant decrease in PTEN that is BCR-ABL1-dependent which accelerates development of a BP phenotype in mice.⁸² Interestingly, it seems that higher levels of BCR-ABL1 are necessary for the non-hypoxic induction of the hypoxia-inducible factor-1 α (HIF-1 α) that, in turn, seems to be required by BP progenitors to tolerate enhanced BCR-ABL1 signaling and to exhibit TKI-resistance *in vivo*.⁸³

The real role of BCR-ABL1 is controversial both in the regulation of survival and in the acquisition of a stem cell phenotype (e.g. self-renewal) by the GMPs. It has been reported that BP GMPs act as LSCs and that acquisition of self-renewal is dependent, at least in part, on BCR-ABL1-dependent induction of β -catenin expression/activity.^{84,85} However, some recent data suggest that BCR-ABL1 activity is actually dispensable for the β -catenin-dependent self-renewal of BP GMPs^{39,68,80,86} and that aberrant modulation of other pathways (e.g. sonic hedgehog, HES1) may also contribute but do not, *per se*, confer “stemness” to BCR-ABL1-positive GMPs.^{67,87} Likewise, recent reports indicate that survival of BP progenitors might occur in a BCR-ABL1-independent manner and involve aberrant expression of pro-survival factors (e.g. BCL2, BCL-XL, MCL1),⁸⁸⁻⁹¹ despite the fact that a plethora of evidence accumulated during the past two decades showed a strict dependence between BCR-ABL1 activity and proliferation/survival signals in CD34⁺ BP progenitors.⁶¹ It was recently reported that targeting BCR-ABL1 expression in the lineage-negative Sca⁺ cell compartment induces a CML-like MPD that in 70% of mice progresses to BP-like disease with lymphoid or myeloid features.³²

These apparent incongruities might depend on the existence in BP of BCR-ABL1-positive progenitors which are capable of originating or maintaining a BP phenotype and that, for still unknown reasons, are not “oncogene addicted” and, therefore, intrinsically resistant both *in vitro* and *in vivo* to TKI treatment. Other data support this hypothesis and perhaps explain the paradoxical short- but not long-term BCR-ABL1-dependence of BP CD34⁺ progenitors in

TKI-treated BP patients. For example, *in vitro* clonogenic or survival assays performed with CD34⁺ BP progenitors exposed to different TKIs never show complete inhibition of leukemic cell growth/survival, and other oncogenic tyrosine kinases (e.g. JAK2, LYN) may regulate and/or overcome BCR-ABL1 expression or activity.^{91,92} Interestingly, inhibition of the activity of the tumor suppressor PP2A plays a pivotal role in controlling BCR-ABL1-positive stem and progenitor cell survival because it impairs not only BCR-ABL1 but also JAK2, LYN and β -catenin activities when pharmacologically or molecularly reactivated.⁹³

(f) What is the role of the second generation TKI in front-line management of patients with CML in first chronic phase?

For patients with CML in CP the introduction of imatinib mesylate in 1998 was an important therapeutic milestone. About 65% of patients treated with imatinib achieve and maintain for seven or more years a complete cytogenetic response (CCyR) and their survival is substantially longer than that achievable with any previous therapies. The adverse events attributable to imatinib are relatively mild and usually manageable.⁹⁴⁻⁹⁶

Conversely this means that one-third of CP patients cannot tolerate imatinib or have a leukemia that proves to be resistant to the drug.⁹⁶ The best characterized mechanism of resistance is the acquisition of mutations in the BCR-ABL1 kinase domain (KD), of which the T315I mutation is the leading example,⁹⁷ although the majority of CP patients resistant to imatinib have no identifiable KD mutation.

Efforts have, therefore, been made to improve the results achieved with the original TKI. Dasatinib and nilotinib may now replace imatinib as first-line therapy on the basis of their efficacy in patients with CML in CP who had failed imatinib. The third new TKI, bosutinib, is not yet licensed but is now in a phase III clinical trial, initial results of which will be reported shortly. In the short term, all three drugs seem valuable for patients who have failed imatinib; about 40-50% of these patients achieve a CCyR.^{98,99}

The initial results of the ENESTnd trial in which nilotinib was compared prospectively with standard dose imatinib, and of the DASISION study comparing dasatinib with imatinib, were reported recently.^{100,101} Both studies were designed for patients with previously untreated CML in CP. Dasatinib and nilotinib both proved better than imatinib. The principal efficacy observations were a higher rate of incidence of CCyR at 12 months, which was achieved at a faster rate, and a reduced rate of progression to advanced phase following 18 months of follow up for the nilotinib study and 12 months for the dasatinib study.

All three drugs of the newer TKI have specific side effects. Thus dasatinib has been associated with pleural effusions, nilotinib with biochemical changes in liver function and bosutinib with gastrointestinal effects, particularly diarrhea. None of these drugs is active for patients in whom the Ph-positive clone consists predominantly of cells with a T315I mutation. Preliminary data suggest that a new multi-kinase inhibitor, ponatinib (previously AP24534), may be able to overcome resistance attributable to the T315I.^{102,103}

There are still a number of unresolved issues in the management of CML in CP. For example, (a) it is not yet clear whether the clinician should leave treatment unchanged in a patient who achieves a complete cytogenetic response

but no major molecular response; (b) it is not yet clear whether the second generation TKIs should be used for all newly diagnosed patients or whether it would be expedient to retain imatinib as initial therapy in some cases; (c) it would be very valuable if one could reliably predict responses in individual patients and design appropriate therapy for those who develop resistance to existing TKIs; and most importantly, (d) we need a therapeutic strategy (other than allogeneic stem cell transplantation) designed to 'cure' CML (see below).

(f) How can we target CML stem cells in responders to TKIs?

CML is recognized as a paradigm for cancer stem cells. Although TKIs have dramatically altered the history of chronic phase CML these drugs are expensive and unavailable to most people with CML who live outside North America or Western Europe. Side effects are substantial and drug-adherence studies suggest overall compliance levels of about 70 percent at two years, with levels of compliance strongly correlated to disease response.^{104,105}

CML cells that originate from a CML stem cell follow a near normal differentiation hierarchy despite the fact that they originate from a leukemia stem cell (LSC). Therapy with TKIs targets the more mature BCR-ABL1⁺ and results in and causes a rapid decline in the numbers of leukemia cells in the blood and bone marrow.^{106,107} This is followed by a much slower reduction in CML progenitor cell numbers over several years. Thereafter, it is unclear whether the slope continues very slowly downwards with cure predicted by 17-20 years¹⁰⁷ or whether disease levels reach a plateau, presumably because at least some CML LSCs are resistant to killing by TKIs. Experimental evidence shows that CML LSCs survive despite continued exposure to TKIs was shown *in vitro*¹⁰⁸⁻¹¹⁰ and *in vivo*.¹¹¹

Major efforts are underway to apply systems biology approaches to compare normal HSC with CML LSC with the goal of finding novel targets that will allow selective eradication of LSCs without harming normal HSCs.^{112,113} Many investigators have already begun pre-clinical work to target LSCs (Table 2). These approaches can be broadly categorized in 3 ways (Table 3): (1) drugs/strategies that induce apoptosis of quiescent LSCs in a selective or non-selective manner; (2) drugs/strategies that address micro-environmental LSC interactions and affect quiescence, self-renewal and/or differentiation; and (3) drugs/strategies focused on immune therapy.

In this rapidly developing field it is difficult to see where the most important advances will occur. Whilst others are focusing on optimizing vaccinations designed to eliminate LSC, the groups of Tessa Holyoake and Ravi Bhatia have studied approaches that target quiescent LSCs directly or aim to drive LSCs into cell-cycle to sensitize them to TKIs. Some of these approaches have been or will be in clinical trials.^{67,137,138}

Autophagy is a cellular lysosomal degradation pathway essential for the regulation of cell survival and death. One of the key regulators of autophagy is mTOR. In normal HSCs, nutrients and growth factors signal via PI3K/AKT/mTOR and keep autophagy suppressed. This pathway is mimicked by BCR-ABL1 signaling in CML LSCs. In normal HSCs, growth factor deprivation results in inhibition of mTOR and induces autophagy. Similarly, when CML LSCs are exposed to TKIs there is potent inhibition of BCR-ABL1 signaling, which induces autophagy. We recently reported that

in CML LSCs this induced autophagy provides a survival mechanism that, at least in part, explains resistance to TKIs.¹⁵⁷ Autophagy inhibitors are being developed but none is currently available for clinical trials. Chloroquine is a potent inhibitor of autophagy. We hypothesized that combining a TKI with chloroquine would shift the balance between apoptosis and induced autophagy in favor of enhanced apoptosis of CML LSCs. This was shown *in vitro* and in animal models and is the basis of a randomized clinical trial comparing imatinib with or without hydroxychloroquine in chronic phase CML patients achieving a major cytogenetic response.

(e) New therapies for Ph-negative MPNs?

The evolving understanding of the molecular pathogenesis of Ph-negative MPNs has led to an unprecedented variety of different therapies. JAK2-inhibitor testing began testing in 2007. These trials focused on persons with MPN-associated myelofibrosis (post-PV MF, post-ET MF and fibrotic PMF). These drugs are now being studied with persons with PV and ET unresponsive to other therapies. Alternative therapeutic strategies against different targets are also being developed.

MPN-associated myelofibrosis

There is no FDA-approved therapy for MPN-associated myelofibrosis. Current therapies are off-label indications palliative for anemia and/or reduction in splenomegaly.¹⁴⁰ Allografts cure some persons with MPN-associated myelofibrosis but are used in fewer than 5% of affected persons.¹⁴¹

JAK2 Inhibitors

Patterns of efficacy and toxicity of JAK2-inhibitors are increasingly overlapping. Drugs in the most advanced phases of testing include INCB18424,¹¹⁴ TG101348,¹¹⁶ SB1518¹¹⁷ and CEP-701¹¹⁸ (Table 2). All rapidly reduce splenomegaly and improve myelofibrosis-associated constitutional symptoms, without significantly changing molecular or histological features of the disease.

Toxicities include anemia and thrombocytopenia. Gastrointestinal toxicity is common amongst drugs inhibiting FLT3.

Alternative targets

The immune-modulatory drug pomalidomide produces durable anemia responses without bone marrow suppression or neuropathy.¹²² The nuclear histone deacetylase inhibitor LBH589 (Novartis) has promising activity in myelofibrosis with responses in splenomegaly and anemia.¹²⁰ A phase II study is ongoing in the USA. The mTOR (mammalian target of rapamycin)-inhibitor RAD001 has activity similar to JAK2-inhibitors (decreased splenomegaly and improved constitutional symptoms).¹²¹

Polycythemia vera and essential thrombocythemia

Therapy of PV and ET relies on short-term anti-platelet drugs¹⁴² and selected cytoreduction for persons at high risk

of vascular events (hydroxycarbamide, anagrelide and alkylating agents).¹⁴³ In PV/ET we still need new drugs (1) for persons resistant or intolerant of current drugs to prevent vascular events; and (2) to delay progression to myelofibrosis and blastic transformation.

JAK-inhibitors

Data on therapy with INCB18424 were reported in 73 PV/ET patients failing hydroxycarbamide.¹¹⁵ There was a complete response in 94% of patients with PV and 61% of patients with ET. No patients had a vascular event on-study.

Splenomegaly and constitutional symptoms improved similar to data in MPN-associated myelofibrosis. Results from a phase II trial with CEP701 in 39 subjects were less impressive with several on-study vascular events and substantial gastrointestinal toxicity.¹¹⁹

Pegylated interferon alpha-2a

Conventional interferon alpha has been used in persons with ET and PV but it is not well-tolerated. Two recent trials show better tolerance, efficacy in preventing vascular events and the potential to produce molecular and histological responses in persons with high-risk ET and PV.^{123;124} This might decrease the likelihood of progression to myelofibrosis and blastic transformation. An international phase III trial comparing hydroxyurea and pegylated interferon alpha-2a is planned.

Participants

The paper follows a small meeting in Natchez, Mississippi, USA immediately after the 2009 American Society of Hematology myeloproliferative neoplasms (MPNs). The organisers invited some discussants to briefly review selected topics addressed at the Natchez meeting and add relevant new data presented or published in 2010. The following persons participated in the meeting: Ralph Arlinghaus (USA), Tiziano Barbui (Italy), Francois Delhommeau (Paris), Connie Eaves (Canada), Alan Gewirtz (USA), John Goldman (UK), Anthony Green (UK), Oliver Hantschel (Austria), Rudiger Hehlmann (Germany), Tessa Holyoake (UK), Brian Huntly (UK), Catriona Jamieson (USA), Steffen Koschmieder (Germany), Robert Kralovics (Austria), Ruben Mesa (US), Tariq Mughal (UK), Vivian Oehler (USA), Heike Pahl (Germany), Animesh Pardanani (USA), Emmanuel Passegue (USA), Danilo Perrotti (USA), Josef Prchal (USA), Giuseppe Saglio (Italy), Richard Silver (USA), Tomasz Skorski (USA), Simona Soverini (Italy), Moshe Talpaz (USA), Richard van Etten (USA), Alessando Vannucchi (Italy), Jean Luc Villeval (France)

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.

References

1. Van Etten RA, Shannon KM. Focus on myeloproliferative diseases and myelodysplastic syndromes. *Cancer Cell*. 2004; 6(6):547-52.
2. Van Etten RA. Models of chronic myeloid leukemia. *Curr Oncol Rep*. 2001;3(3):228-37.
3. Lacout C, Pisani DF, Tulliez M, Gachelin FM, Vainchenker W, Villeval JL. JAK2V617F expression in murine hematopoietic cells leads to MPD mimicking human PV with secondary myelofibrosis. *Blood*. 2006;

- 108(5):1652-60.
4. Wernig G, Mercher T, Okabe R, Levine RL, Lee BH, Gilliland DG. Expression of Jak2V617F causes a polycythemia vera-like disease with associated myelofibrosis in a murine bone marrow transplant model. *Blood*. 2006;107(11):4274-81.
 5. Zaleskas VM, Krause DS, Lazarides K, Patel N, Hu Y, Li S, Van Etten RA. Molecular pathogenesis and therapy of polycythemia induced in mice by JAK2 V617F. *PLoS One*. 2006;1:e18.
 6. Bumm TC, Elsea C, Corbin AS, Loriaux M, Sherbenou D, Wood L, et al. Characterization of murine JAK2V617F-positive myeloproliferative disease. *Cancer Res*. 2006;66(23):1156-65.
 7. Scott LM, Tong W, Levine RL, Scott MA, Beer PA, Stratton MR, et al. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. *N Engl J Med*. 2007;356(5):459-68.
 8. Pikman Y, Lee BH, Mercher T, McDowell E, Ebert BL, Gozo M, et al. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. *PLoS Med*. 2006;3(7):e270.
 9. Akada H, Yan D, Zou H, Hutchison RE, Mohi MG. Conditional Expression of Heterozygous or Homozygous Jak2V617F From Its Endogenous Promoter Induces a Polycythemia Vera-Like Disease. *ASH Annual Meeting Abstracts 2009;114(22):437*.
 10. Ghevaert CJ, Li J, Severin S, Auger J, Watson S, Green AR. Physiological Levels of Jak2 V617F Result in Enhanced Megakaryocyte Differentiation, Proplatelet Formation and Platelet Reactivity. *ASH Annual Meeting Abstracts 2009;114(22):226*.
 11. Marty C, Lacout C, Fong L, Martin A, Vainchenker W, Villevall JL. Constitutive Heterozygous Expression of JAK2V617F Mutated Kinase Triggers Severe Myeloproliferative Disorder in Knock-in Mice. *ASH Annual Meeting Abstracts 2009;114(22):2902*.
 12. Tiedt R, Hao-Shen H, Sobas MA, Looser R, Dirnhofer S, Schwaller J, Skoda RC. Ratio of mutant JAK2-V617F to wild-type Jak2 determines the MPD phenotypes in transgenic mice. *Blood*. 2008;111(8):3931-40.
 13. Shide K, Shimoda HK, Kumano T, Karube K, Kameda T, Takenaka K, et al. Development of ET, primary myelofibrosis and PV in mice expressing JAK2 V617F. *Leukemia*. 2008;22(1):87-95.
 14. Xing S, Wanting TH, Zhao W, Ma J, Wang S, Xu X, et al. Transgenic expression of JAK2V617F causes myeloproliferative disorders in mice. *Blood*. 2008;111(10):5109-17.
 15. Akada H, Yan D, Zou H, Fiering S, Hutchison RE, Mohi MG. Conditional expression of heterozygous or homozygous Jak2V617F from its endogenous promoter induces a polycythemia vera-like disease. *Blood*. 2010;115(17):3589-97.
 16. Mullally A, Lane SW, Ball B, Megerdichian C, Okabe R, Al Shahrouf F, et al. Physiological Jak2V617F expression causes a lethal myeloproliferative neoplasm with differential effects on hematopoietic stem and progenitor cells. *Cancer Cell*. 2010;17(6):584-96.
 17. Lu X, Levine R, Tong W, Wernig G, Pikman Y, Zamegar S, et al. Expression of a homodimeric type I cytokine receptor is required for JAK2V617F-mediated transformation. *Proc Natl Acad Sci USA*. 2005;102(52):18962-7.
 18. James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature*. 2005;434(7037):1144-8.
 19. Heisterkamp N, Jenster G, Kioussis D, Pattengale PK, Groffen J. Human bcr-abl gene has a lethal effect on embryogenesis. *Transgenic Res*. 1991;1(1):45-53.
 20. Kralovics R, Teo SS, Li S, Theocharides A, Buser AS, Tichelli A, Skoda RC. Acquisition of the V617F mutation of JAK2 is a late genetic event in a subset of patients with myeloproliferative disorders. *Blood*. 2006;108(4):1377-80.
 21. Scott LM, Scott MA, Campbell PJ, Green AR. Progenitors homozygous for the V617F mutation occur in most patients with polycythemia vera, but not essential thrombocythemia. *Blood*. 2006;108(7):2435-7.
 22. Bellanne-Chantelot C, Chaumarel I, Labopin M, Bellanger F, Barbu V, De Toma C, et al. Genetic and clinical implications of the Val617Phe JAK2 mutation in 72 families with myeloproliferative disorders. *Blood*. 2006;108(1):346-52.
 23. Dazzi F, Capelli D, Hasserjian R, Cotter F, Corbo M, Poletti A, et al. The kinetics and extent of engraftment of chronic myelogenous leukemia cells in non-obese diabetic/severe combined immunodeficiency mice reflect the phase of the donor's disease: an in vivo model of chronic myelogenous leukemia biology. *Blood*. 1998;92(4):1390-6.
 24. Eisterer W, Jiang X, Christ O, Glimm H, Lee KH, Pang E, et al. Different subsets of primary chronic myeloid leukemia stem cells engraft immunodeficient mice and produce a model of the human disease. *Leukemia*. 2005;19(3):435-41.
 25. Daley GQ, Van Etten RA, Baltimore D. Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. *Science*. 1990;247(4944):824-30.
 26. Gishizky ML, Johnson-White J, Witte ON. Efficient transplantation of BCR-ABL-induced chronic myelogenous leukemia-like syndrome in mice. *Proc Natl Acad Sci USA*. 1993;90(8):3755-9.
 27. Li S, Ilaria RL Jr, Million RF, Daley GQ, Van Etten RA. The P190, P210, and P230 forms of the BCR/ABL oncogene induce a similar chronic myeloid leukemia-like syndrome in mice but have different lymphoid leukemogenic activity. *J Exp Med*. 1999;189(9):1399-412.
 28. Hu Y, Swerdlow S, Duffy TM, Weinmann R, Lee FY, Li S. Targeting multiple kinase pathways in leukemic progenitors and stem cells is essential for improved treatment of Ph+ leukemia in mice. *Proc Natl Acad Sci USA*. 2006;103(45):16870-5.
 29. Van Etten RA. Studying the pathogenesis of BCR-ABL+ leukemia in mice. *Oncogene*. 2002;21(56):8643-51.
 30. Miyazaki K, Yamasaki N, Oda H, Kuwata T, Kanno Y, Miyazaki M, et al. Enhanced expression of p210BCR/ABL and aberrant expression of Zfp423/ZNF423 induce blast crisis of chronic myelogenous leukemia. *Blood*. 2009;113(19):4702-10.
 31. Nagamachi A, Yamasaki N, Miyazaki K, Oda H, Miyazaki M, Honda Z, et al. Haploinsufficiency and acquired loss of Bcl11b and H2AX induces blast crisis of chronic myelogenous leukemia in a transgenic mouse model. *Cancer Sci*. 2009;100(7):1219-26.
 32. Perez-Caro M, Cobaleda C, Gonzalez-Herrero I, Vicente-Duenas C, Bermejo-Rodriguez C, Sanchez-Beato M, et al. Cancer induction by restriction of oncogene expression to the stem cell compartment. *EMBO J*. 2009;28(1):8-20.
 33. Koschmieder S, Gottgens B, Zhang P, Iwasaki-Arai J, Akashi K, Kutok JL, et al. Inducible chronic phase of myeloid leukemia with expansion of hematopoietic stem cells in a transgenic model of BCR-ABL leukemogenesis. *Blood*. 2005;105(1):324-34.
 34. Schemionek M, Elling C, Steidl U, Baumer N, Hamilton A, Spieker T, et al. BCR-ABL enhances differentiation of long-term repopulating hematopoietic stem cells. *Blood*. 2010;115(16):3185-95.
 35. Li J, Spensberger D, Ahn JS, Anand S, Beer PA, Ghevaert C, et al. JAK2 V617F impairs hematopoietic stem cell function in a conditional knock-in mouse model of JAK2 V617F-positive essential thrombocythemia. *Blood*. 2010;116(9):1528-38.
 36. Zhang B, Ho YW, Lee SU, Maeda T, Huettner C, Bhatia R. Characterization of Leukemia-Initiating Cells in a Transgenic Model of Chronic Phase Chronic Myelogenous Leukemia (CML). *ASH Annual Meeting Abstracts 2009;114(22):858*.
 37. Zhang B, Strauss AC, Chu S, Li M, Ho Y, Shiang KD, et al. Effective Targeting of Quiescent Chronic Myelogenous Leukemia Stem Cells by Histone Deacetylase Inhibitors in Combination with Imatinib Mesylate. *Cancer Cell*. 2010;17(5):427-42.
 38. Harb J, Neviani P, Huettner C, Marcucci G, Perotti D. BCR/ABL Dosage Hierarchically and Temporally Influences hnRNP A1, hnRNP K and hnRNP E2 Expression in Hematopoietic Stem and Progenitor Cells. *ASH Annual Meeting Abstracts 2009;114(22):191*.
 39. Oaks J, Neviani P, Mukhopadhyay A, Santhanam R, Ma Y, Mao C, et al. FTY720 but Not Its Immunosuppressive Phosphorylated Form FTY720-P Exerts Anti-Leukemic Activity towards Ph(+) and Ph(-) Myeloproliferative Disorders through Reactivation of the PP2A Tumor Suppressor. *ASH Annual Meeting Abstracts 2009;114(22):3259*.
 40. Sengupta A, Arnett J, Dunn S, Williams DA, Cancelas JA. Rac2 GTPase deficiency depletes BCR-ABL+ leukemic stem cells and progenitors in vivo. *Blood*. 2010;116(1):81-4.
 41. Nussenzveig RH, Swierczek SI, Jelinek J, Gaikwad A, Liu E, Verstovsek S, et al. Polycythemia vera is not initiated by JAK2V617F mutation. *Exp Hematol*. 2007;35(1):32-8.
 42. Levine RL, Belisle C, Wadleigh M, Zahrieh D, Lee S, Chagnon P, et al. X-inactivation-based clonality analysis and quantitative JAK2V617F assessment reveal a strong association between clonality and JAK2V617F in PV but not ET/MMM, and identifies a subset of JAK2V617F-negative ET and MMM patients with clonal hematopoiesis. *Blood*. 2006;107(10):4139-41.
 43. Campbell PJ, Baxter EJ, Beer PA, Scott LM, Bench AJ, Huntly BJ, et al. Mutation of JAK2 in the myeloproliferative disorders: timing, clonality studies, cytogenetic associations, and role in leukemic transformation. *Blood*. 2006;108(10):3548-55.
 44. Schaub FX, Jager R, Looser R, Hao-Shen H, Hermouet S, Girodon F, et al. Clonal analysis of deletions on chromosome 20q and JAK2-V617F in MPD suggests that del20q acts independently and is not one of the predisposing mutations for JAK2-V617F. *Blood*. 2009;113(9):2022-7.
 45. Theocharides A, Boissinot M, Girodon F,

- Garand R, Teo SS, Lippert E, et al. Leukemic blasts in transformed JAK2-V617F-positive myeloproliferative disorders are frequently negative for the JAK2-V617F mutation. *Blood*. 2007;110(1):375-9.
46. Carbuccia N, Murati A, Trouplin V, Brecqueville M, Adelaide J, Rey J, et al. Mutations of ASXL1 gene in myeloproliferative neoplasms. *Leukemia*. 2009;23(11):2183-6.
47. Delhommeau F, Dupont S, Della Valle V, James C, Trannoy S, Massé A, et al. Mutation in TET2 in myeloid cancers. *N Engl J Med*. 2009;360(22):2289-301.
48. Gelsi-Boyer V, Trouplin V, Adelaide J, Bonansea J, Cervera N, Carbuccia N, et al. Mutations of polycomb-associated gene ASXL1 in myelodysplastic syndromes and chronic myelomonocytic leukaemia. *Br J Haematol*. 2009;145(6):788-800.
49. Langemeijer SM, Kuiper RP, Berends M, Knops R, Aslanyan MG, Massop M, et al. Acquired mutations in TET2 are common in myelodysplastic syndromes. *Nat Genet*. 2009;41(7):838-42.
50. Ito S, D'Alessio AC, Taranova OV, Hong K, Sowers LC, Zhang Y. Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. *Nature*. 2010;466(7310):1129-33.
51. Beer PA, Delhommeau F, LeCouedic JP, Dawson MA, Chen E, Bareford D, et al. Two routes to leukemic transformation after a JAK2 mutation-positive myeloproliferative neoplasm. *Blood*. 2010;115(14):2891-900.
52. Saint-Martin C, Leroy G, Delhommeau F, Panelatti G, Dupont S, James C, et al. Analysis of the ten-eleven translocation 2 (TET2) gene in familial myeloproliferative neoplasms. *Blood*. 2009;114(8):1628-32.
53. Swierczek S, Bellanne-Chantelot C, Yoon D, Saint-Martin C, Kim SJ, Najman A, Prchal JT. TET2 Mutations in Polycythemia Vera (PV) in Some Cases Follow Rather Than Precede JAK2 V617F Mutation, Are Not a Disease-Initiating Event, Affect Mainly Erythropoiesis, and Contribute to Increased Aggressivity of PV Clone. *ASH Annual Meeting Abstracts* 2009;114(22):3913.
54. Abdel-Wahab O, Manshoury T, Patel J, Harris K, Yao J, Hedvat C, et al. Genetic analysis of transforming events that convert chronic myeloproliferative neoplasms to leukemias. *Cancer Res*. 2010;70(2):447-52.
55. Aslanyan MG, Langemeijer SMC, Cilloni D, Saglio G, Marie JP, Tang R, et al. Incidence and Clinical Impact of TET2 Mutations in Acute Myeloid Leukemia Patients Treated within the EORTC/GIMEMA AML-12/06991 AML Trial. *ASH Annual Meeting Abstracts* 2009;114(22):2609.
56. Campbell PJ. Somatic and germline genetics at the JAK2 locus. *Nat Genet*. 2009;41(4):385-6.
57. Jones AV, Chase A, Silver RT, Oscier D, Zoi K, Wang YL, et al. JAK2 haplotype is a major risk factor for the development of myeloproliferative neoplasms. *Nat Genet*. 2009;41(4):446-9.
58. Kilpivaara O, Mukherjee S, Schram AM, Wadleigh M, Mullally A, Ebert BL, et al. A germline JAK2 SNP is associated with predisposition to the development of JAK2(V617F)-positive myeloproliferative neoplasms. *Nat Genet*. 2009;41(4):455-9.
59. Olcaydu D, Harutyunyan A, Jager R, Berg T, Gisslinger B, Pabinger I, et al. A common JAK2 haplotype confers susceptibility to myeloproliferative neoplasms. *Nat Genet*. 2009;41(4):450-4.
60. Cross NCP, Campbell P, Beer PA, Schnittger S, Vannucchi AM, Zoi K, et al. The JAK2 46/1 Haplotype Predisposes to Myeloproliferative Neoplasms Characterized by Diverse Mutations. *ASH Annual Meeting Abstracts* 2009;114(22):433.
61. Perrotti D, Jamieson C, Goldman J, Skorski T. Chronic myeloid leukemia: mechanisms of blastic transformation. *J Clin Invest*. 2010;120(7):2254-64.
62. Nicholson E, Holyoake T. The chronic myeloid leukemia stem cell. *Clin Lymphoma Myeloma* 2009;9(Suppl 4):S376-S381.
63. Nieborowska-Skorska M, Koptyra M, Bolton E, Ray R, Ngaba D, Hoser G, et al. ROS-Induced DNA Damage Causing Genomic Instability in CML Stem and/or Progenitor Cells and in Quiescent and/or Proliferating Cells: Role of Mitochondrial Respiratory Chain Complex III. *Blood* (ASH Annual Meeting Abstracts) 2009;114(22):3268.
64. Hu Y, Chen Y, Douglas L, Li S. beta-Catenin is essential for survival of leukemic stem cells insensitive to kinase inhibition in mice with BCR-ABL-induced chronic myeloid leukemia. *Leukemia* 2009;23(1):109-16.
65. Neviani P, Santhanam R, Ma Y, Marcucci G, Byrd JC, Chen CS, et al. Activation of PP2A by FTY720 Inhibits Survival and Self-Renewal of the Ph(+) Chronic Myelogenous Leukemia (CML) CD34+/CD38- Stem Cell through the Simultaneous Suppression of BCR/ABL and BCR/ABL-independent Signals. *Blood* (ASH Annual Meeting Abstracts) 2008;112(1):189.
66. Zhao C, Chen A, Jamieson CH, Fereshteh M, Abrahamsson A, Blum J, et al. Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia. *Nature*. 2009;458(7239):776-9.
67. Dierks C, Beigi R, Guo GR, Zirlirk K, Stegert MR, Manley P, et al. Expansion of Bcr-Abl-positive leukemic stem cells is dependent on Hedgehog pathway activation. *Cancer Cell*. 2008;14(3):238-49.
68. Peterson LE, Turbiak AJ, Giannola DM, Donato N, Showalter HHD, Fearon ER, Talpaz M. Wnt-Pathway Directed Compound Targets Blast Crisis and Chronic Phase CML Leukemia Stem Progenitors. *Blood* (ASH Annual Meeting Abstracts) 2009;114(22):2168.
69. Irvine DA, Zhang B, Allan EK, Holyoake TL, Dorsch M, Manley PW, et al. Combination of the Hedgehog Pathway Inhibitor LDE225 and Nilotinib Eliminates Chronic Myeloid Leukemia Stem and Progenitor Cells. *Blood* (ASH Annual Meeting Abstracts) 2009;114(22):1428.
70. Nagao R, Ashihara E, Kimura S, Yao H, Takeuchi M, Yokota A, et al. Inhibition of Wnt/(beta)-Catenin Signaling by AV65 Treatment Caused Cell Cycle Arrest and Induced Caspase-Independent or -Dependent Apoptosis in CML Cells. *Blood* (ASH Annual Meeting Abstracts) 2009;114(22):2184.
71. Cilloni D, Pellicano F, Helgason VG, Panuzzo C, Messa F, Huntly BJ, et al. Foxo Transcription Factor Activity Is Retained in Quiescent Chronic Myeloid Leukemia Stem Cells and Activated by Tyrosine Kinase Inhibitors to Mediate "induced-quiescence" in More Mature progenitors. *Blood* (ASH Annual Meeting Abstracts) 2009;114(22):187.
72. Sengupta A, Arnett J, Dunn S, Cancelas J. Bmi-1 Overexpression Synergizes with p210-BCR-ABL to Induce Stem Cell and Progenitor Transformation. *ASH Annual Meeting Abstracts* 2009;114(22):3251.
73. Sengupta A, Arnett J, Dunn S, Cancelas J. Deletion of Rac2 inhibits Proliferation of Chronic Myelogenous Leukemia (CML) Stem Cells and Progenitors (HSC/P) In Vivo and Promotes Survival of Scl/p210-BCR-ABL Mice. *ASH Annual Meeting Abstracts* 2009;114(22):3253.
74. Hurtz C, Duy C, Cerchietti L, Park E, Ci W, Swaminathan S, et al. BCL6 Is Required for Leukemia-Initiation and Self-Renewal Signaling in Chronic Myeloid Leukemia. *ASH Annual Meeting Abstracts* 2009;114(22):2167.
75. Takeuchi M, Kimura S, Kuroda J, Ashihara E, Kawatani M, Osada H, et al. Hypoxia-Adapted CML Cells Are More Primitive Population and Are Eradicated by Glyoxalase-1 Inhibitors. *ASH Annual Meeting Abstracts* 2009;114(22):2166.
76. Ng KP, Poh TY, Sun WT, Chuah C, Ong ST. Physiologic Hypoxia Protects Chronic Myelogenous Leukemia Progenitors From Elimination by Imatinib Mesylate. *ASH Annual Meeting Abstracts* 2009;114(22):2181.
77. Klemm L, Duy C, Iacobucci I, von Levetzow G, Feldhahn N, Kim Ym, et al. The B Cell Mutator AID Promotes B Lymphoid Blast Crisis and Drug-Resistance in Chronic Myeloid Leukemia. *ASH Annual Meeting Abstracts* 2009;114(22):3274.
78. Mullighan CG, Miller CB, Radtke I, Phillips LA, Dalton J, Ma J, et al. BCR-ABL1 lymphoblastic leukaemia is characterized by the deletion of Ikaros. *Nature* 2008;453(7191):110-4.
79. Iacobucci I, Storlazzi CT, Cilloni D, Lonetti A, Ottaviani E, Soverini S, et al. Identification and molecular characterization of recurrent genomic deletions on 7p12 in the IKZF1 gene in a large cohort of BCR-ABL1-positive acute lymphoblastic leukemia patients: on behalf of Gruppo Italiano Malattie Ematologiche dell'Adulto Acute Leukemia Working Party (GIMEMA AL WP). *Blood*. 2009;114(10):2159-67.
80. Neviani P, Santhanam R, Trotta R, Notari M, Blaser BW, Liu S, et al. The tumor suppressor PP2A is functionally inactivated in blast crisis CML through the inhibitory activity of the BCR/ABL-regulated SET protein. *Cancer Cell*. 2005; 8(5):355-68.
81. Eiring AM, Harb JG, Neviani P, Garton C, Oaks JJ, Spizzo R, et al. miR-328 functions as an RNA decoy to modulate hnRNP E2 regulation of mRNA translation in leukemic blasts. *Cell*. 2010;140(5):652-65.
82. Peng C, Chen Y, Yang Z, Zhang H, Osterby L, Rosmarin AG, Li S. PTEN is a tumor suppressor in CML stem cells and BCR-ABL-induced leukemias in mice. *Blood*. 2010; 115(3):626-35.
83. Zhao F, Mancuso A, Bui TV, Tong X, Gruber JJ, Swider CR, et al. Imatinib resistance associated with BCR-ABL upregulation is dependent on HIF-1alpha-induced metabolic reprogramming. *Oncogene* 2010;29(20):2962-72.
84. Jamieson CH. Chronic myeloid leukemia stem cells. *Hematology Am Soc Hematol Educ Program*. 2008;436-42.
85. Jamieson CH, Ailles LE, Dylla SJ, Muijtjens M, Jones C, Zehnder JL, et al. Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. *N Engl J Med*. 2004;351(7):657-67.
86. Lim S, Saw TY, Chuah C, Ong ST. Overexpression and Phosphorylation of eIF4E Is Required for Beta-Catenin Activation in Blast Crisis Chronic

- Myelogenous Leukemia. *ASH Annual Meeting Abstracts* 2009;114(22):41.
87. Nakahara F, Sakata-Yanagimoto M, Komeno Y, Kato N, Uchida T, Haraguchi K, et al. Hes1 immortalizes committed progenitors and plays a role in blast crisis transition in chronic myelogenous leukemia. *Blood*. 2010;115(14):2872-81.
 88. Goff D, Abrahamsson-Schairer A, Shih A, Black JM, Chuang R, Tesi RJ, et al. The Broad Spectrum Bcl-2 Inhibitor Apogossypol Induces Apoptosis and Differentiation of Blast Crisis Chronic Myeloid Leukemia Stem Cells. *ASH Annual Meeting Abstracts* 2009;114(22):3275.
 89. Mak DH, Schober WD, Konopleva M, Cortes J, Kantarjian HM, Andreeff M, Carter BZ. Inhibition of Bcl-2/Bcl-XL Promotes Apoptosis in Blast Crisis CML Including Quiescent Primitive Progenitor Cells Regardless of Cellular Responses to Tyrosine Kinase Inhibitors. *ASH Annual Meeting Abstracts* 2009;114(22):646.
 90. Belloc F, Airiau K, Jeanneteau M, Mahon FX. ABT-737 Cooperates in a Strong Synergism with Tyrosine Kinase Inhibitors to Induce Apoptosis of Chronic Myeloid Leukemia Cells. *ASH Annual Meeting Abstracts* 2009;114(22):3246.
 91. Pasquet JM, Gioia R, Drullion C, Lagarde V, Leroy C, Roche S, et al. Tyrosine Kinase Proteins profiling of Nilotinib Resistant Chronic Myelogenous Leukemia Cells Unravels a Tyrosine Kinase-Mediated Bypass. *ASH Annual Meeting Abstracts* 2009;114(22):2175.
 92. Samanta AK, Chakraborty SN, Sun X, Schlette E, Priebe W, Arlinghaus RB. Jak2 Phosphorylates Tyr 177 of Bcr-Abl Activating the Ras and PI-3 Kinase Pathways and Maintains Functional Levels of Bcr-Abl in Chronic Myelogenous Leukemia. *ASH Annual Meeting Abstracts* 2009;114(22):39.
 93. Perrotti D, Neviani P. Protein phosphatase 2A (PP2A), a drugable tumor suppressor in Ph1(+) leukemias. *Cancer Metastasis Rev*. 2008;27(2):159-68.
 94. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med*. 2001;344(14):1031-7.
 95. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med*. 2003;348(11):994-1004.
 96. Mughal TI, Schrieber A. Principal long-term adverse effects of imatinib in patients with chronic myeloid leukemia in chronic phase. *Biologics*. 2010;4:315-23.
 97. Shah NP, Nicoll JM, Nagar B, Gorre ME, Paquette RL, Kuriyan J, Sawyers CL. Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. *Cancer Cell*. 2002;2(2):117-25.
 98. Talpaz M, Shah NP, Kantarjian H, Donato N, Nicoll J, Paquette R, et al. Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. *N Engl J Med*. 2006;354(24):2531-41.
 99. Kantarjian HM, Giles F, Gattermann N, Bhalla K, Alimena G, Palandri F, et al. Nilotinib (formerly AMN107), a highly selective BCR-ABL tyrosine kinase inhibitor, is effective in patients with Philadelphia chromosome-positive chronic myelogenous leukemia in chronic phase following imatinib resistance and intolerance. *Blood*. 2007;110(10):3540-6.
 100. Kantarjian H, Shah NP, Hochhaus A, Cortes J, Shah S, Ayala M, et al. Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med*. 2010;362(24):2260-70.
 101. Saglio G, Kim DW, Issaragrisil S, le Coutre P, Etienne G, Lobo C, et al. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. *N Engl J Med*. 2010;362(24):2251-9.
 102. O'Hare T, Shakespeare WC, Zhu X, Eide CA, Rivera VM, Wang F, et al. AP24534, a pan-BCR-ABL inhibitor for chronic myeloid leukemia, potently inhibits the T315I mutant and overcomes mutation-based resistance. *Cancer Cell* 2009;16(5):401-12.
 103. Cortes J, Talpaz M, Deininger M, Shah N, Flinn IW, Mauro MJ, et al. A Phase 1 Trial of Oral AP24534 in Patients with Refractory Chronic Myeloid Leukemia and Other Hematologic Malignancies: First Results of Safety and Clinical Activity against T315I and Resistant Mutations. *ASH Annual Meeting Abstracts* 2009;114(22):643.
 104. Noens L, van Lierde MA, De Bock R, Verhoef G, Zachee P, Bememan Z, et al. Prevalence, determinants, and outcomes of nonadherence to imatinib therapy in patients with chronic myeloid leukemia: the ADAGIO study. *Blood*. 2009;113(22):5401-11.
 105. Marin D, Bazeos A, Mahon FX, Eliasson L, Milojkovic D, Bua M, et al. Adherence is the critical factor for achieving molecular responses in patients with chronic myeloid leukemia who achieve complete cytogenetic responses on imatinib. *J Clin Oncol*. 2010;28(14):2381-8.
 106. Michor F, Hughes TP, Iwasa Y, Branford S, Shah NP, Sawyers CL, Nowak MA. Dynamics of chronic myeloid leukaemia. *Nature* 2005;435(7046):1267-70.
 107. Roeder I, Horn M, Glauche I, Hochhaus A, Mueller MC, Loeffler M. Dynamic modeling of imatinib-treated chronic myeloid leukemia: functional insights and clinical implications. *Nat Med*. 2006;12(10):1181-4.
 108. Graham SM, Jorgensen HG, Allan E, Pearson C, Alcorn MJ, Richmond L, Holyoake TL. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. *Blood*. 2002;99(1):319-25.
 109. Jorgensen HG, Allan EK, Jordanides NE, Mountford JC, Holyoake TL. Nilotinib exerts equipotent antiproliferative effects to imatinib and does not induce apoptosis in CD34+ CML cells. *Blood*. 2007;109(9):4016-9.
 110. Copland M, Hamilton A, Elrick LJ, Baird JW, Allan EK, Jordanides N, et al. Dasatinib (BMS-354825) targets an earlier progenitor population than imatinib in primary CML but does not eliminate the quiescent fraction. *Blood*. 2006;107(11):4532-9.
 111. Chu S, Lin A, McDonald T, Snyder DS, Forman SJ, Bhatia R. Persistence of Leukemia Stem Cells in Chronic Myelogenous Leukemia Patients in Complete Cytogenetic Remission on Imatinib Treatment for 5 Years. *Blood* (ASH Annual Meeting Abstracts) 2008;112(11):194.
 112. Graham SM, Vass JK, Holyoake TL, Graham GJ. Transcriptional analysis of quiescent and proliferating CD34+ human hemopoietic cells from normal and chronic myeloid leukemia sources. *Stem Cells*. 2007;25(12):3111-20.
 113. Griffiths SD, Burthem J, Unwin RD, Holyoake TL, Melo JV, Lucas GS, Whetton AD. The use of isobaric tag peptide labeling (iTRAQ) and mass spectrometry to examine rare, primitive hematopoietic cells from patients with chronic myeloid leukemia. *Mol Biotechnol*. 2007;36(2):81-9.
 114. Verstovsek S, Kantarjian H, Mesa RA, Pardanani AD, Cortes-Franco J, Thomas DA, et al. Safety and efficacy of INCB018424, a JAK1 and JAK2 inhibitor, in myelofibrosis. *N Engl J Med*. 2010;363(12):1117-27.
 115. Verstovsek S, Passamonti F, Rambaldi A, Barosi G, Rosen P, Levy R, et al. A Phase 2 Study of INCB018424, An Oral, Selective JAK1/JAK2 Inhibitor, in Patients with Advanced Polycythemia Vera (PV) and Essential Thrombocythemia (ET) Refractory to Hydroxyurea. *Blood* (ASH Annual Meeting Abstracts) 2009;114(22):311.
 116. Pardanani AD, Gotlib JR, Jamieson C, Cortes J, Talpaz M, Stone R, et al. A Phase I Evaluation of TG101348, a Selective JAK2 Inhibitor, in Myelofibrosis: Clinical Response Is Accompanied by Significant Reduction in JAK2V617F Allele Burden. *Blood* (ASH Annual Meeting Abstracts) 2009;114(22):755.
 117. Verstovsek S, Odenike O, Scott B, Estrov Z, Cortes J, Thomas DA, Wood J, Ethirajulu K, Lowe A, Zhu HJ, Kantarjian H, Deeg HJ. Phase I Dose-Escalation Trial of SB1518, a Novel JAK2/FLT3 Inhibitor, in Acute and Chronic Myeloid Diseases, Including Primary or Post-Essential Thrombocythemia/ Polycythemia Vera Myelofibrosis. *Blood* (ASH Annual Meeting Abstracts) 2009;114(22):3905.
 118. Hexner E, Goldberger JD, Prchal JT, Demakos EP, Swierczek S, Weinberg RS, et al. A Multicenter, Open Label Phase I/II Study of CEP701 (Lestaurtinib) in Adults with Myelofibrosis; a Report On Phase I: A Study of the Myeloproliferative Disorders Research Consortium (MPD-RC). *Blood* (ASH Annual Meeting Abstracts) 2009;114(22):754.
 119. Moliterno AR, Hexner E, Roboz GJ, Carroll M, Luger S, Mascarenhas J, et al. An Open-Label Study of CEP-701 in Patients with JAK2 V617F-Positive PV and ET: Update of 39 Enrolled Patients. *Blood* (ASH Annual Meeting Abstracts) 2009;114(22):753.
 120. Mascarenhas J, Wang X, Rodriguez A, Xu M, Gorman E, Zhang W, et al. A Phase I Study of LBH589, a Novel Histone Deacetylase Inhibitor in Patients with Primary Myelofibrosis (PMF) and Post-Polycythemia/Essential Thrombocythemia Myelofibrosis (Post-PV/ET MF). *Blood* (ASH Annual Meeting Abstracts) 2009;114(22):308.
 121. Vannucchi AM, Guglielmelli P, Gattoni E, Antonioli E, Bogani C, Tozzi L, et al. RAD001, An Inhibitor of mTOR, Shows Clinical Activity in a Phase I/II Study in Patients with Primary Myelofibrosis (PMF) and Post Polycythemia Vera/Essential Thrombocythemia Myelofibrosis (PPV/PET MF). *Blood* (ASH Annual Meeting Abstracts) 2009;114(22):307.
 122. Mesa RA, Pardanani AD, Hussein K, Wu W, Schwager S, Litzow MR, et al. Phase I/2 study of Pomalidomide in myelofibrosis. *Am J Hematol*. 2010;85(2):129-30.
 123. Kiladjian JJ, Cassinat B, Chevret S, Turlure P, Cambier N, Roussel M, et al. Pegylated interferon-alfa-2a induces complete hematologic and molecular responses with low

- toxicity in polycythemia vera. *Blood*. 2008; 112(8):3065-72.
124. Quintas-Cardama A, Kantarjian H, Manshouri T, Luthra R, Estrov Z, Pierce S, et al. Pegylated interferon alfa-2a yields high rates of hematologic and molecular response in patients with advanced essential thrombocythemia and polycythemia vera. *J Clin Oncol*. 2009;27(32):5418-24.
 125. Copland M, Pellicano F, Richmond L, Allan EK, Hamilton A, Lee FY, et al. BMS-214662 potently induces apoptosis of chronic myeloid leukemia stem and progenitor cells and synergizes with tyrosine kinase inhibitors. *Blood*. 2008;111(5):2843-53.
 126. Pellicano F, Copland M, Jorgensen HG, Mountford J, Leber B, Holyoake TL. BMS-214662 induces mitochondrial apoptosis in chronic myeloid leukemia (CML) stem/progenitor cells, including CD34+38-cells, through activation of protein kinase Cbeta. *Blood*. 2009;114(19):4186-96.
 127. Heaney NB, Copland M, Stewart K, Godden J, Parker AN, McQuaker IG, et al. Complete molecular responses are achieved after reduced intensity stem cell transplantation and donor lymphocyte infusion in chronic myeloid leukemia. *Blood* 2008; 111(10):5252-5.
 128. Olavarria E, Siddique S, Griffiths MJ, Avery S, Byrne JL, Piper KP, et al. Posttransplantation imatinib as a strategy to postpone the requirement for immunotherapy in patients undergoing reduced-intensity allografts for chronic myeloid leukemia. *Blood*. 2007;110(13):4614-7.
 129. Bocchia M, Gentili S, Abruzzese E, Fanelli A, Iuliano F, Tabilio A, et al. Effect of a p210 multipeptide vaccine associated with imatinib or interferon in patients with chronic myeloid leukaemia and persistent residual disease: a multicentre observational trial. *Lancet*. 2005;365(9460):657-62.
 130. Rojas JM, Knight K, Wang L, Clark RE. Clinical evaluation of BCR-ABL peptide immunisation in chronic myeloid leukaemia: results of the EPIC study. *Leukemia*. 2007;21(11):2287-95.
 131. Rezvani K, Yong AS, Tawab A, Jafarpour B, Eniafe R, Mielke S, et al. Ex vivo characterization of polyclonal memory CD8+ T-cell responses to PRAME-specific peptides in patients with acute lymphoblastic leukemia and acute and chronic myeloid leukemia. *Blood*. 2009;113(10):2245-55.
 132. Yong AS, Keyvanfar K, Hensel N, Eniafe R, Savani BN, Berg M, et al. Primitive quiescent CD34+ cells in chronic myeloid leukemia are targeted by in vitro expanded natural killer cells, which are functionally enhanced by bortezomib. *Blood*. 2009; 113(4):875-82.
 133. Heaney NB, Pellicano F, Zhang B, Crawford L, Chu S, Kazmi SM, et al. Bortezomib induces apoptosis in primitive chronic myeloid leukemia cells including LTC-IC and NOD/SCID repopulating cells. *Blood*. 2010;115(11):2241-50.
 134. Jagani Z, Song K, Kutok JL, Dewar MR, Melet A, Santos T, et al. Proteasome inhibition causes regression of leukemia and abrogates BCR-ABL-induced evasion of apoptosis in part through regulation of forkhead tumor suppressors. *Cancer Res*. 2009; 69(16):6546-55.
 135. Hu Z, Pan XF, Wu FQ, Ma LY, Liu DP, Liu Y, et al. Synergy between proteasome inhibitors and imatinib mesylate in chronic myeloid leukemia. *PLoS One*. 2009; 4(7):e6257.
 136. Bellodi C, Lidonnici MR, Hamilton A, Helgason GV, Soliera AR, Ronchetti M, et al. Targeting autophagy potentiates tyrosine kinase inhibitor-induced cell death in Philadelphia chromosome-positive cells, including primary CML stem cells. *J Clin Invest*. 2009;119(5):1109-23.
 137. Jorgensen HG, Copland M, Allan EK, Jiang X, Eaves A, Eaves C, Holyoake TL. Intermittent exposure of primitive quiescent chronic myeloid leukemia cells to granulocyte-colony stimulating factor in vitro promotes their elimination by imatinib mesylate. *Clin Cancer Res*. 2006;12(2):626-33.
 138. Foo J, Drummond MW, Clarkson B, Holyoake T, Michor F. Eradication of chronic myeloid leukemia stem cells: a novel mathematical model predicts no therapeutic benefit of adding G-CSF to imatinib. *PLoS Comput Biol*. 2009;5(9):e1000503.
 139. Drummond MW, Heaney N, Kaeda J, Nicolini FE, Clark RE, Wilson G, et al. A pilot study of continuous imatinib vs pulsed imatinib with or without G-CSF in CML patients who have achieved a complete cytogenetic response. *Leukemia*. 2009;23(6):1199-201.
 140. Mesa RA. How I treat symptomatic splenomegaly in patients with myelofibrosis. *Blood*. 2009;113(22):5394-400.
 141. Kroger N, Holler E, Kobbe G, Bornhauser M, Schwerdtfeger R, Baumann H, et al. Allogeneic stem cell transplantation after reduced-intensity conditioning in patients with myelofibrosis: a prospective, multicenter study of the Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation. *Blood*. 2009;114(26):5264-70.
 142. Landolfi R, Marchioli R, Kutti J, Gisslinger H, Tognoni G, Patrono C, Barbui T. Efficacy and safety of low-dose aspirin in polycythemia vera. *N Engl J Med*. 2004; 350(2):114-24.
 143. Harrison CN, Campbell PJ, Buck G, Wheatley K, East CL, Bareford D, et al. Hydroxyurea compared with anagrelide in high-risk essential thrombocythemia. *N Engl J Med*. 2005;353(1):33-45.