

News in cytogenetic and molecular diagnosis TEL-JAK-2 fusion as a model for hybrid oncogenes

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ABSTRACT

Chronic myeloproliferative disorders represent a heterogeneous group of hematological neoplasias with origin at multi-potent stem cell level, of which the molecular pathogenesis, with the exception of chronic myeloid leukemia, was less understood until now. By identifying chromosomal reorganizations that generate gene fusion, which code tyrosin kinases, the cytogenetic and molecular studies made progresses in decoding the pathogenic mechanisms of negative-BCR/ABL chronic myeloproliferative disorders. The recent identification of a punctual mutation that determines a constitutive activation of Jak2 kinases, also with activation of ulterior signaling pathways, starts to be considered the key genetic event in the majority of patients with chronic malignant myeloproliferative syndromes. In this context JAK2 becomes a promising candidate-gene for decoding the pathogenesis of maladies such as Polycythemia Vera (PV), Essential Thrombocythemia (TE) and Myelofibrosis Myeloid Metaplasia (MMM). The molecular oriented therapy uses a new generation of cancer active drugs, destined to interfere with the molecular target with critical role in tumour initiation or progression. The identification of proper molecular targets is possible only through detailed deciphering of carcinogenesis mechanisms. Modern therapy in chronic mieloproliferative syndromes brings upfront drugs, which target oncogenic tyrosin-kinases, which are fusion oncoproteins, generated by chromosomal reorganizations in negative-BCR/ABL chronic myeloproliferative disorders, which thus become ideal targets for these strategies. The tyrosin-kinases most frequently involved in the pathogenesis of chronic myeloproliferative disorders are Jak2, Abl, Kit, PDGFRB, and PDGFRA. Inactivation of these kinases leads to rapid apoptosis of malignant cells.

During the last decade major progresses were recorded worldwide regarding the understanding of the molecular events regulating the hematopoietic differentiation and the aberrant intracellular signaling due to the alteration of the growth factor receptors. The multidisciplinary

approach and the permanent information exchange concerning the clinical aspects, cellular and molecular genetics are mandatory for advancing in the understanding of leukemia biology, identification of new therapeutic targets and in proving the anti-leukemic efficiency of potentially new therapies. The chronic myeloproliferative disorders represent a heterogeneous

Used shortcuts:

ALL – Acute lymphoblastic leukemia

CML – Chronic myelocytic leukemia

IL – Interleukin

G-CSF – Growth-colony stimulatory factor

Jak – Janus kinases

TEL – Translocation ETS Leukemia (ETV6)

PI3'kinaza – phosphatidyil inositol 3' kinases

PKB – protein kinases B

STAT – signal transducers and activators of transcription

PDGFbR – platelet-derived growth factor receptor b gene

FISH – Fluorescence In Situ Hybridization

group of hematological neoplastic disorders originating in the multi-potent stem cells. Besides the four entities initially included, the chronic granulocytic leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis myeloid metaplasia (MMM), which all form the classical chronic myeloproliferative syndrome (SMP), some other conditions were also included. They have been classified as chronic atypical myeloproliferative disorders: atypical myeloid leukemia, the hypereosinophilic syndrome (HES), chronic leukemia with eosinophils, chronic leukemia with neutrophils (LCN), chronic myelomonocytic leukemia (CMML), systemic mastocytosis (SM), juvenile myelomonocytic leukemia (JMML). Unlike the CML where the value of the Philadelphia chromosome as a genetic marker of the disease has proven to be beyond the molecular pathogenesis of all chronic myeloproliferative disorders BCR/ABL-negative, was so far scarcely understood. During the past several years, numerous cytogenetic and molecular biology studies suggested that a single somatic mutation of Jak-2 might be responsible for the myeloproliferative disorders and for some of the acute lymphoproliferative disorders (especially lymphoblastic). This discovery might have a huge impact on the diagnosis and treatment of patients (3). This way, the abnormalities of STAT 3, 5, the increase of Bcl xl and the increase of PKB/AKT activity subsequent to the mutations involving the Jak-2, like the translocation of t (9;15;12) (p24; q15; p13) might be relevant to the chronic myeloproliferative disorders (4); the Tel – Jak-2 fusion in the t translocation (9;12) (p24;p13) is determinant in the induction of ALL, while the Tel – PDGF-R β fusion in the t translocation (5;17) – to the chronic myelomonocytic leukemia. The role of the disturbances in the activity of the JAK-2 kinase in the numerous malignant hematological disorders was recently revised. Large studies have proven that:

- 97% of the patients with PV presented Jak-2 somatic mutations, which led to a cell line hypersensitive to erythropoietin, as well as to a cellular survival independent of the growth factors; the agents interfering with the Jak-2 inhibit the formation of erythroid colonies in patients with PV(1,2,8);
- 57% of the patients with ET showed Jak-2 mutations (3,4);

- 89% of the patients with CGL also present with a Tel-Jak-2 fusion as part of the t translocation (9;15;12) (p24; q15; p13) (3,4);
- 50% of the patients with MMM present with Jak-2 somatic mutations (4).

A punctual mutation of JAK-2 – V617F has been recently identified in a significant number of the patients suffering from chronic myeloproliferative disorders (89% PV, 43% ET and 43% MMM) (1-4). More and more of the studies seem to prove that this mutation determining the constitutive activation of Jak-2-kinase and the activation of the subsequent signaling pathways represent the key genetic event in most of the patients with chronic malignant myeloproliferative disorders (5-8). In this context JAK-2 represents a promising candidate-gene for deciphering the pathogenesis of PV, ET and MMM.

The genes participating in the translocations that determine malignant hematological disorders usually encode the tyrosine-kinase, transcription factors and factors capable of altering transcription processes.

The prototype for the alterations in genes encoding enzymes capable of tyrosine-kinase-like activities is the bcr-abl translocation. The substrates activated by this tyrosine kinase are: STAT 1,3,5,6; Grb 2 – sos – and they can be either directly or indirectly recruited via ship 1,2, shc, cbc while also synchronously activating the signaling pathway of phosphatidylinositol 3' kinase (PI 3' kinase) (12).

The prototype for the translocations involving transcription factors are the Tel fusions (ets transcription factors) with the PDGF-R beta and abl – two tyrosine kinases the constitutional activation of which is mediated by this translocation. There are numerous subsequent translocations associated to the constitutive activation of the receptor tyrosine kinase PDGF-R beta, some of the ones usually quoted in the specialized literature being: t (5;7) (q33; q11), t (5;10) (q33; q21), t (5;17) (q33; p13), t (5;14) (q33; q32), t (1;5) (q23; q33), t (5;17) (q33; p11) (9). These translocations are specifically associated to a BCR / ABL negative chronic myeloproliferative subtype – CMML. The PDGFR beta gene rearrangements usually associated with the atypical chronic myeloid leukemia – HES are accomplished via various cytogenic reorganization processes: translocations [t(4;22)(q12; q11), t(1;4)(q44; q12)], interstitial deletions (10,11).

TEL – JAK-2 is another fusion – but still requires further study – that might represent a model for the molecular characterization of the chronic myeloproliferative disorders. The TEL-JAK-2 expression protects cells against apoptosis and is usually involved in the resistance to the imatinib mesylate (Gleevec) molecular therapy.

Jak-2: the Jak-2 gene is located on the 9 p23-p24 chromosome. The Jak-2 messenger RNA is condensed in the spleen, the lymph nodes and in the peripheral blood lymphocytes; lower concentrations are usually encountered in the thymus and within the bone marrow, tissues that are quite rich in young and immature lymphocytes. The Jak-2 concentration increases in the B lymphocytes subsequent to mitogenic or anti-IgM stimulation, and up to significantly lower levels into the stimulated T-lymphocytes. Moreover, the high Jak-2 levels were depicted in the pre-B leukemic cells. (15)

Jak-s are intra-cytoplasmic tyrosine kinases involved in signaling to the nucleus the information acquired from specific surface cell receptors, especially from cytokine receptors, lacking the intrinsic tyrosine-kinase activation. There are four JAKs: JAK 1, 2, 3 and TYK2. The Jak kinase structure is unique, featuring three critical domains:

- JH1, the tyrosine kinase domain, is located towards the carboxyl ending of the protein;

- JH2, the pseudo-kinase domain, is similar to the JH1 domain, but lacks however, the tyrosine-kinase activity;
- The FERM domain (4 point one, Ezrin, Radixin, Moesin), located in the immediate proximity of the amino ending of the protein, is responsible for the non-covalent bonding to “box1/motifs”, a radical in the immediate proximity of the cytoplasmic membrane region, next to the cytokine receptor #1.

These cytokine receptors connect to the growth factors (EPO, SCF, GM-CSF, IL-3, TPO, IGF-1) to which the hematopoietic progenitors are hypersensitive to.

The binding of a ligand to the cytokine receptor results in the activation of Jak – namely of the JH1 domain – which leads to the phosphorylation of the tyrosine, thus creating connectivity sites for SH2 (src homology 2) which further binds signaling molecules – like the STAT (Shc, Gab/IRS) – or regulating subunits of the kinase – like the p85 in PI 3' kinase (15).

The JAK proteins mediate all cytokine effects in the hematopoietic cells via the activation of the STAT and of the phosphatidylinositol 3-kinase, via the phosphorylation of the PKB/Akt, via inducing the expression of bcl-2, via stimulating the RAS-MAPK pathway, via inducing the c-fos and c-myc genes etc.

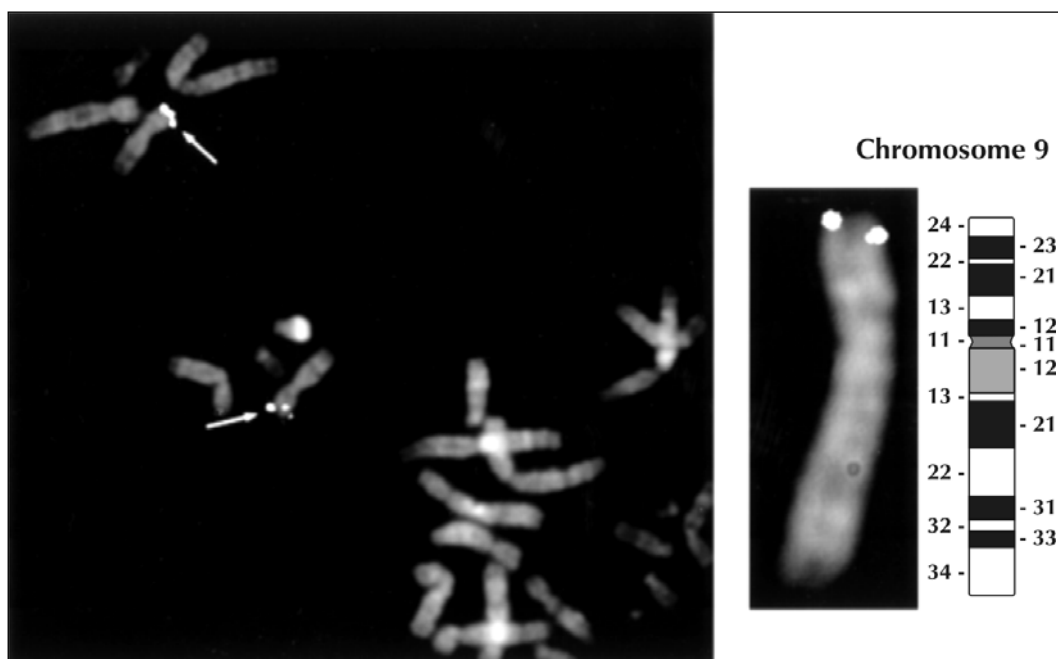


FIGURE 1. Chromosome localization of the Jak-2 human gene; positional mapping acquired using FISH during the chromosome metaphases of normal human lymphocytes (15)

TEL: the TEL gene is situated on the 12 p13 chromosome and is also known as the Ets-translocation variant gene 6 (ETV-6). It also occurs as a fusion partner in numerous translocations involved in the leukemia genesis (for instance with PDGF- β R in CGL with t (5;12) (q31; p13), with AML1 in pre-B LAL with t (12;21) (p13; q22), with abl in LAL with t (9;12) (q34; p13), with MDS / EVI1 in the myeloproliferative disorders with t (3;12) (q26; p13) etc. The TEL gene encodes a nuclear protein scarcely expressed but which includes a preserved DNA-linkage domain at the carboxyl ending of the protein – a domain belonging to the Ets transcription factors. TEL represents a subset of the family of Ets transcription factors which also includes an amino ending, bearing multiple names (PNT – pointed domain, NCR – N-terminal conserved domain, helix-loop-helix domain). This PNT is extremely important to the transcriptional activity (16).

TEL merges with either the kinase domain JH1 or the pseudo-kinase domain JH2 (merging with JH2 + JH1) and thus generates an active JAK molecule. The helix-loop-helix (HLH) domain of the TEL transcription factor merges with the JH1/JH2 catalytic domain of JAK-2, which increases the kinase activity and the involvement of the nucleus-incoming signaling pathways (STAT; PI 3' kinase and PKB) (19).

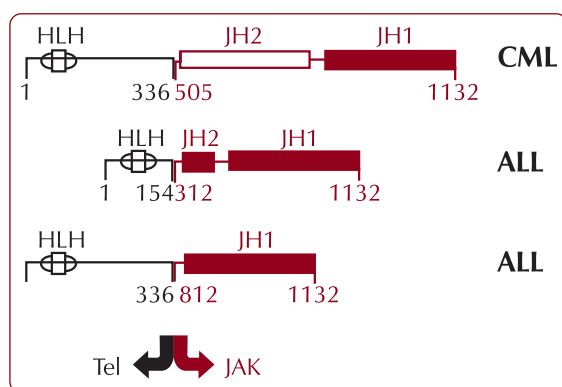


FIGURE 2. The oligomerization domain of the TEL transcription factor is fused with the JH1 catalytic domain of JAK-2 either directly or indirectly via the JH2 (19)

There are three types of mergers:

1. t (9;12) (p24; p13) TEL JAK-2 (4;17); this means that the fourth exon of TEL merges with the seventeenth exon of JAK-2 in pre-B LAL;

2. t (9;12) (p24; p13) TEL JAK-2 (5;19); this means that the fifth exon of TEL merges with the nineteenth exon of JAK-2 in LAL with the T cell;
3. t (9;15;12) (p24; q15; p13); this means that the fifth exon of TEL merges with the twelfth exon of JAK-2 frequently in atypical CGL.

The TEL JAK-2 fusion becomes oncogenic *in vivo* and fully capable of altering the IL-3 – dependent cell lines into cell series independent of the growth factors, thus being involved in the leukemia genesis process.

During the TEL-Jak-2 fusion, the Jak-2 tyrosine kinase is constitutively activated as a result of the oligomerization of the TEL PNT domain; this process results in the alteration of the hematopoietic cell line.

This fusion quite frequently determines the bi-phenotype disorder combining myeloproliferative and lymph proliferative components. It is frequently encountered in pre-B cell LAL with and quite rarely in T-cell LAL, in those LAL with a severe prognosis and especially in atypical forms of chronic myeloid leukemia (especially in blast attacks dominated by lymphoblast cells) as well as in selected severe-diagnosis LAL (19).

A brief presentation of the mechanisms involved in the leukemia genesis process via TEL-JAK-2 fusion:

STAT activation:

- signaling mechanism for the JAK STAT pathway;
- the result of JAK STAT pathway activation:(12,17,18)
- the intrinsic tyrosine kinase activity phosphorylated various substrates, including STAT 1,3,5 which leads to the transformation of the cell line depending on the growth factors (especially on IL-3) into a cellular line independent of the activity of growth factors;
- the STAT activation can also be randomly, JAK-2-independently initiated (within the cells with a JAK-2 / TYK2 deficit, G-CSF may activate STAT independently of the JAK activation, while there can only be a partial reduction of the STAT-3 activity) in the cells with insufficient JAK1. TEL – JAK-2 is constitutively phosphorylated and cannot phosphorylate the endogenous JAK, so that the activation of the constitutive JAK within the malignant cells is no

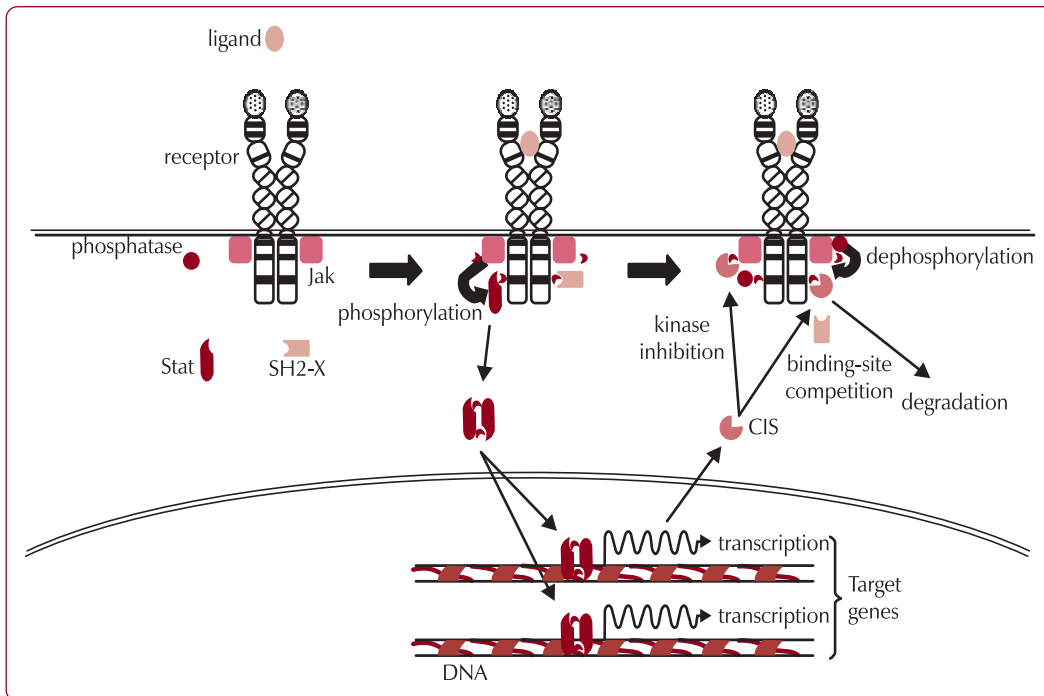


FIGURE 3. When a ligand joins the cytokine receptor with no tyrosine kinase activity, the auto-phosphorylation of the tyrosine is initiated, leading to the JAK-2 activation. The JAK activation induces the phosphorylation of the receptor, creating linkage sites for specific signal-proteins, including the STAT proteins further phosphorylated by JAK using the remaining tyrosine kinase preserved at the carboxyl ending of the protein. The STAT proteins then form stable homo-dimers and hetero-dimers thanks to the interaction between the SH2 domain (src homology 2) of a STAT protein and the phosphor-tyrosine of another. It further undergoes a translocation into the nucleus where it alters the transcription of target-genes by influencing the specific regulating sequences (12).

longer required; the JAK-negative cells are incapable to inhibit the STAT-5 activation or the growth factor – independent proliferation within cells hosting a TEL-JAK fusion. Shortly, the TEL – JAK fusion increases the tyrosine kinase activity, phosphorylates the constitutive tyrosine and activates the STAT-5 which later on undergoes translocation into the nucleus (where it links to the DNA and alters the transcription of the target-genes). However, the lack of STAT-5 within the cells results in the inability to inhibit the resistance to apoptosis, to inhibit the growth-factor-independent proliferation and to inhibit the potential for developing leukemia within the cells which are altered by the presence of the above-mentioned translocation (12,17,18).

Activation of the phosphatidylinositol-3'-kinase signal pathway (PI 3' kinase)

The PI 3' kinase features two subunits: subunit p110 (the catalytic subunit), and subunit

p85, (the regulating subunit); due to the regulating subunit the PI 3' kinase is capable of linking to the JAK-2. The activation of the catalytic subunit p110 in the structure of the PI 3' kinase depends on the association of the regulating subunit p85 with the activated tyrosine kinases. It is a well-known fact that the bcr – abl activates the PI 3' kinase after bonding with p85; it is also a proven fact that TEL-JAK-2 is capable of interacting with the p85 subunit as well. This regulating subunit of the PI 3' kinase comprises two SH2 domains and one SH3 domain, since the TEL-JAK-2 – p85 interaction is SH2-dependent. (20) The link between PI 3' kinase and TEL-JAK-2, mediated by the p85 subunit, requires:

- One linear amino acid group– p85 binding motif-in TEL-JAK-2 serving as a linkage site for the p85 protein into a region maintained as a tyrosine kinase domain of the first receptor of the fibroblastic growth factor (this site was found to be important in the activation of the PI 3' kinase following the activation of the fibroblastic growth factor receptor).

All of the TEL-JAK-2 fusion proteins contain one YMIM amino acid group, which might generate this p85-binding motif.

Another protein named Gab 2 protein that might represent a potential mediator in the association between the PI 3' kinase and the TEL-JAK-2. The involvement of an adaptive molecule is widely spread between all known signaling pathways. The direct recruitment of p85 – TEL-JAK-2 via the linkage site and YMIM as a linkage site are insignificant, hence it was suggested that there might be an adaptive molecule responsible for mediating the association between TEL-JAK-2 and p85 and also that this molecule could be Gab 2. The TEL-JAK-2 – Gab 2 association was proven to be independent regarding to the IL-3 stimulation. Gab 2 is constitutively tyrosine-phosphorylated under basal conditions. TEL-JAK-2 stimulates the tyrosine-phosphorylation while the IL-3 stimulation induces the slowest Gab 2 migration, as IL-3 stimulates the association of shp-2 and shc with Gab 2 (12,17,18).

The PI 3' kinase pathway activates the phosphorylation of PKB (protein kinase B) constitutive/Akt. The growth factors and the cytokines (especially IL-3) induce the activation of the PI 3' kinase signaling pathways. The mergers involving tyrosine kinases activate the PI 3' kinase, as PKB is the main component of this pathway. This way the cellular survival is regulated. It was also proven that IL-3 would induce an intense PKB phosphorylation and that TEL-JAK-2 would induce an increase of the PKB phosphorylation even in the lack of IL-3. Down regulation of the PI 3' kinase activity via the pharmacological inhibitor Ly 294002 would then induce the inhibition of the cellular growth (in the cell lines presenting the TEL-JAK-2 fusion) even if IL-3 is present. This way both the growth induced by TEL-JAK-2 and the PKB phosphorylation are blocked without altering the STAT-5 pathways. Hence it became a proven fact that the two pathways, STAT-5 and PKB are independent signaling pathways (12,17,18).

Other functions of JAK-2:

- The expression of JAK-2 induces bcl-2, thus inhibiting the apoptosis (the activation of STAT-3 also induces apoptosis resistance, but via inducing bcl-xl instead);
- Activates the Pyk-2 kinase;

- Stimulates the RAS-MAPK1 pathway;
- Induces the genes for c-fos and c-myc (21).

Inactivation of the Jak-2 signaling pathway:

- The ligand-receptor complex is affected after the activation of the Jak pathway, namely the internalization, which reduces the additional signaling of this pathway;
- The phosphatases associated with the receptor remove the phosphate residuum. A very important role is held by SHP1 (hematopoietic cell phosphatase) whose genetic elimination induces myeloproliferative disorders;
- The activation of STAT during the transmission of the information towards the nucleus results in the transcriptional activation of the SOCS family of proteins (suppressor of cytokine signaling). SOCS links with Jak or with the activated phosphor-tyrosine residuum of the cytokine receptor, blocking any ulterior signaling. □

CONCLUSION

In this context it becomes important to find an answer to the question: How can a single somatic mutation affecting the Jak-2 gene result in several types of malignant blood disorders? There are currently several theories:

- The accumulation of additional genetic alterations, not yet detected;
- The mutations occur in different subpopulations of primitive hematopoietic cells, thus the conversion towards either one of the malignant blood disorders occurs;
- The mutant kinase features particular biochemical properties which enable it to activate a particular cell line, within specific cells this way leading to its uncontrolled proliferation.

The human leukemia represents a paradigm in the diagnostic and treatment of cancer, at the sametime being the first neoplastic disorders in which the effectiveness of molecular-targeted therapies has proven the importance of identifying the underlying pathogenic defects; that is why the treatment of leukemia in humans became one of the best-developed branches of oncology.

The molecular characterization of leukemia became a basic concern for numerous working groups aiming at inserting the results of fundamental research into the clinical practice in order to attain a more accurate diagnosis, to elaborate molecular classifications and to optimize the existing therapies. Translocations and associated molecular abnormalities, identified in various subtypes of BCR/ABL-negative myeloproliferative disorders directs towards the idea that the rearranged and dysfunctional genes encoding the receptor and non-receptor tyrosine kinase would represent an important pathogenic mechanism for chronic leukemic disorders. The dysfunctional tyrosine-kinase of the fusion proteins (meaning exacerbated functionality) would represent an abnormal response to cytokines. The accumulation of new information about the molecular mechanisms initiating and regulating the cytokine activities intensely supports the theory that the chronic myeloproliferative disorders would actually represent disorders based on an altered transmission of cytokine-induced signaling (14). The identification of chromosomal reorganization and characterization of such alterations at a molecular level resulted in valuable information about the genes and genome regions involved in the generation of such neoplastic disorders. The numerous chromosomal reorganizations yet remain unidentified. The on-going efforts to characterize

the molecular lesions using modern techniques are expected to lead to the identification of new genetic defects, leading the way to new therapeutic strategies. The modern chronic myeloproliferative disorder therapies currently focuses on drugs targeting the oncogenic tyrosine-kinases. The fusion proteins within tumors resulting from the chromosomal reorganization in chronic BCR/ABL negative myeloproliferative disorders actually representing the ideal targets for such strategies. The tyrosine kinases most frequently involved in the pathogenicity of the chronic myeloproliferative disorders are Jak-2, Abl, Kit, PDGFRB, PDGFRA. The inactivation of these kinases results in a quick apoptosis of the malignant cells. The success of tyrosine-kinase-inhibitor therapies of the Abl type (for instance, Glivec) in CGL intensified the preoccupation for identifying the malignant blood disorders depending on tyrosine kinases and implicitly responding to Glivec therapy. Glivec (imatinib mesylate) is a 2-phenyl-amino-pyrimidine specifically inhibiting the non-receptor Abl tyrosine kinases (cAbl, vAbl and Bcr-Abl) as well as the receptor tyrosine kinases (c-kit or PDGF receptors). The Glivec therapy has already been successfully used with CMML and HES. However some studies revealed that the imatinib does not inhibit the tyrosine kinase activation of the TEL/Jak-2 fusion compound (9,10,11). □

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