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NEW TRENDS IN THE TREATMENT OF MYELOID MALIGNANCIES

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Summary

Molecular defects discovered in the recent years could explain the pathogenesis of myeloid malignancies. Moreover these alterations serve as the targets for the therapy. In this article treatment approach of myeloid malignancies is presented, together with the new treatment possibilities specifically acting on the level of molecular defects. Therapy with the combination of several agents which target more than one molecular alterations i.e. gene mutation, signal transduction pathway or antigenic determinant may be the most promising therapy in the future.

KEY WORDS: *myeloproliferative disorders, AML, CML, MDS, molecular defects, targeted therapy*

NOVOSTI U LIJEČENJU ZLOĆUDNIH BOLESTI MIJELOIDNIH STANICA

Sažetak

Nedavno otkrivenim molekularnim oštećenjima moguće je objasniti patogenezu zloćudnih bolesti mijeloidnih stanica. Te su promjene usto i ciljevi prema kojima se usmjerava terapija. U ovom članku prikazan je pristup u liječenju zloćudnih bolesti mijeloidnih stanica te nove mogućnosti liječenja s djelovanjem na razini molekularnih oštećenja. Terapija kombinacijom nekoliko lijekova koji ciljaju više od jedne molekularne promjene, npr. gensku mutaciju, prijenos signala ili antigensku determinantu najviše obećava i mogla bi u budućnosti dati najbolje rezultate.

KLJUČNE RIJEČI: *mijeloproliferatvine bolesti, AML, KML, MDS, molekularna oštećenja, ciljana terapija*

INTRODUCTION

The new insights of pathogenesis in myeloid malignancies at the molecular level open a new possibility for therapy. Based on this approaches it is expected in the near future to switch from non-specific standard chemotherapy combined with radiotherapy to more specific treatment. The principle of such therapy is the use of "smart" drugs that act at the level of molecular alterations of myeloproliferation. Some data suggests that targeted therapy could be given alone or in combination with some other treatment approach especially immunotherapy.

In this review article current therapy of myeloproliferative disorders (MPD) will be discussed. Some of the new insight into the molecular basis of the malignant disorders of myeloid progenitors will be presented. The molecular alterations as possible targets for the current and future therapy will be comment.

The terms myeloid malignancies refers to a group of well defined hematopoietic neoplasm involving cells committed to myeloid line to cellular development (Fig. 1.). All of these malignancies show clonal proliferation of myeloid precursors (1, 2).

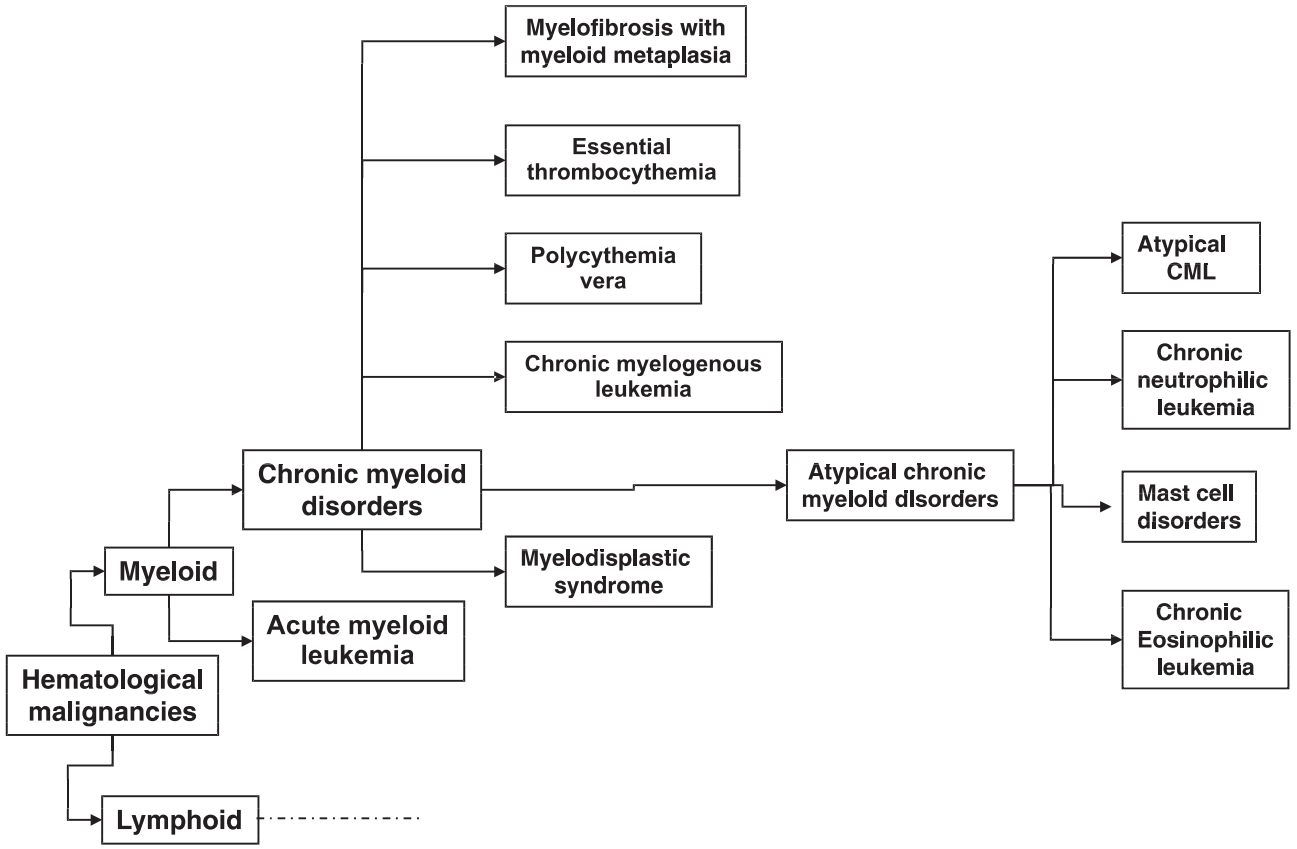

Figure 1. Classification of myeloid malignancies

ACUTE MYELOID LEUKEMIA (AML):

The basic approach of current therapy in AML is to achieve complete remission (CR) of the disease with the rapid restoration of normal bone marrow function. If the patients are not treated after the CR most of them will relapse. Standard postinduction or postremission therapy usually comprises one or more courses of intensive chemotherapy with or without stem cell transplantation. The goal of such therapy is to eradicate residual leukemia, allowing the possibility of cure. During the last three decade combination of cytarabine with daunorubicin (7+3) has been well established as a common remission induction regimen (3). According to the risk for relapse as a postremission therapy patients receive either intensive consolidation or intensive chemotherapy with autologous or allogeneic stem cell transplantation (4, 5). Current results show CR rate in younger adults (< 60 years of age) of 65-80% and overall survival rate of only 30-35% (6). In patients older than 60 years the CR rate is between 40-50%, but there are few (10-15%) long-term survivors (7). It was expected that intensification of therapy by using the higher doses of anthracyclines or cytarabine, addition of other cytotoxic agents such as etoposide, or novel agents with unique mechanisms of action such as the purine analogs might increase antileukemic efficacy (8-11). Preliminary data shows that high-dose cytarabine is effective (CR rate > 80%) with acceptable toxicity (12).

The new treatment strategy for AML depends predominantly on prognostic and risk factors recognized at diagnosis (Fig. 2).

More than 60% of good risk patients receiving chemotherapy with or without autologous transplantation are long term survivors (13). For intermediate and high risk patients allogeneic stem cell transplantation is at the moment the treatment of choice (14, 15).

Moreover, better understanding of AML especially the molecular basis of leukemic cell al-

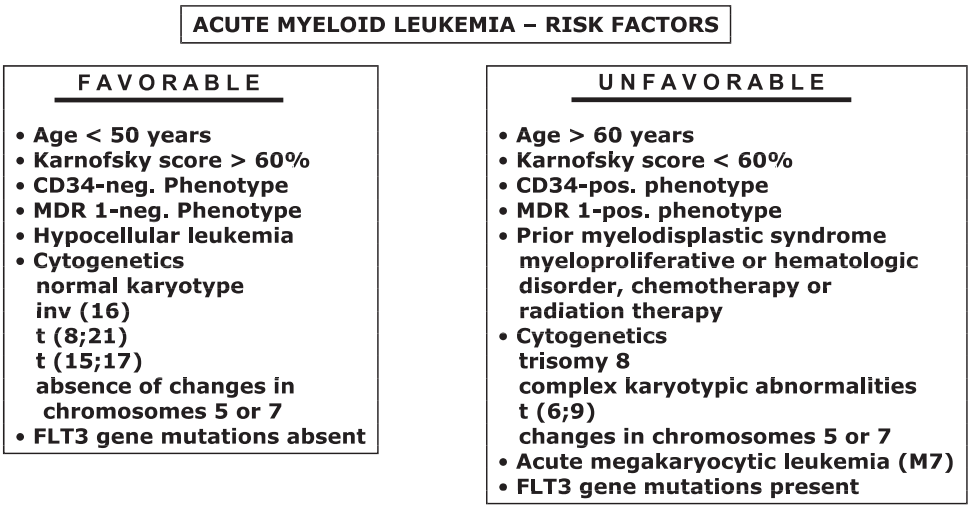

Figure 2. Risk factors in AML

terations (Fig.3.) could explain the pathogenesis of leukemia and might substantially change the prognostic and treatment approach in the near future.

Molecular alterations that disrupt almost every facet of cell transformation could be involved in leukemogensis. These processes include the regulation of cell proliferation, differ-

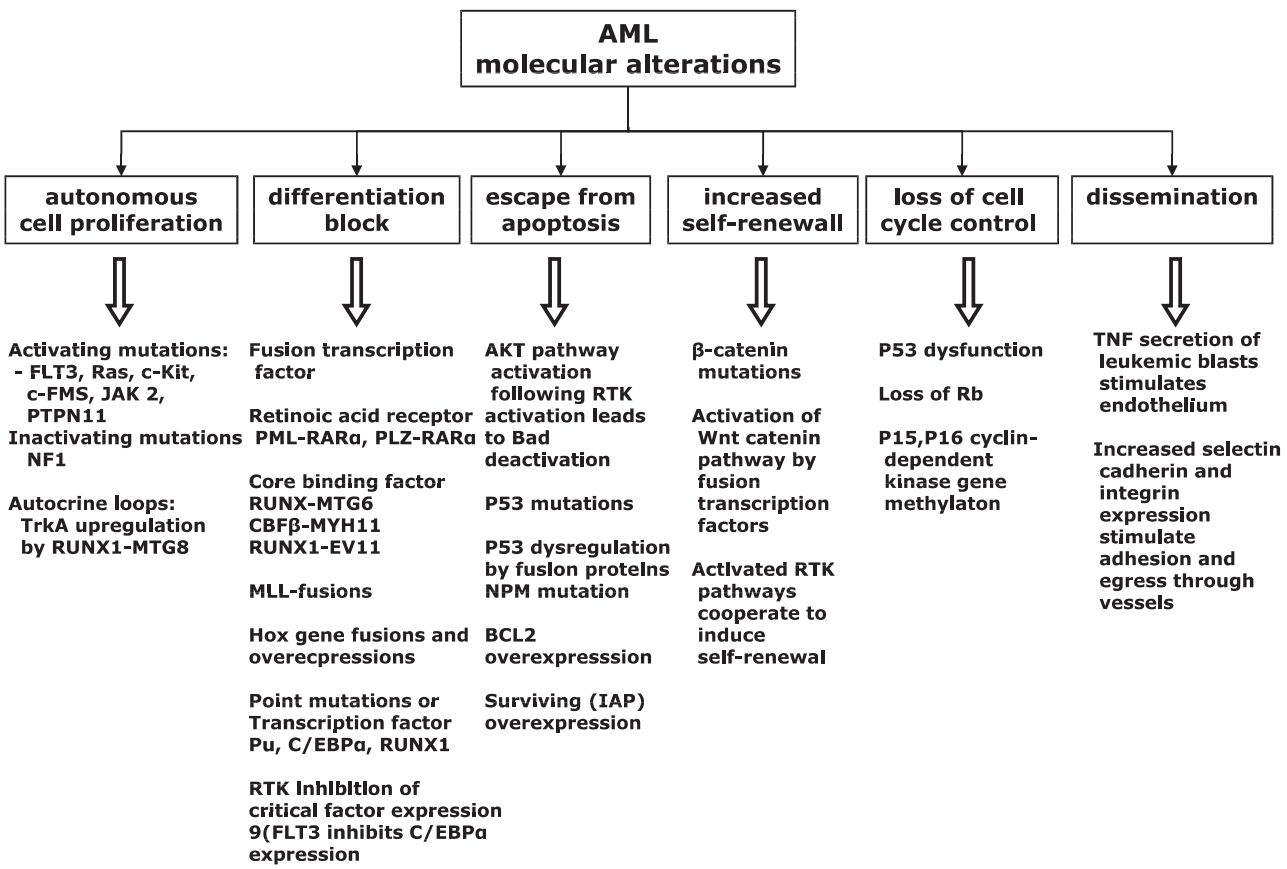

Figure 3. Molecular defects in AML

entiation, self-renewal, survival, cell cycle checkpoint control, DNA repair and chromatin stability, and cell dissemination (see Fig.3.). Some of these alterations are the targets for therapy. It has to be stated that the molecular pathogenesis of AML is complex. In some cases it is obvious that there is a direct correlation between the molecular defect and the biological feature of leukemia, while in the others explanation for the molecular basis of leukemia is still not clear. Better understanding of this links more specific therapy can be developed. Currently the investigational approach is to prove that targeted drugs might influence differentiation block and proliferative activity of leukemic cells, might induce specific apoptosis of malignant cells and might restore cell cycle control.

The new agents in clinical trials in AML are presented in Table 1.

Gemtuzumab ozogamicin is monoclonal anti-CD33 antibody chemically linked to a potent cytotoxic agent, calichemicin. The monoclonal antibody after linkage to CD-33 positive leukemic blasts release calichemicin which disrupt cell organelles ultimately leading to cell death. This drug currently is under investigation in most large cooperative groups in phase II/III studies. In EORTC group older patients are currently randomizing to receive chemotherapy with or without gemtuzumab ozogamomycin and than again randomizing in CR receiving maintenance therapy with or without gemtuzumab ozogamomicin (16). Preliminary data showed that gemtuzumab ozogamomicin given in older patients alone and in combination with chemotherapy could achieve CR rate in 20% and 50% of patients respectively (17). The toxicity risk is acceptable and occasional patients have developed a veno-occlusive disease-like syndrome. For younger patients the preliminary data is encouraging; CR rate with chemotherapy and gemtuzumab ozogamomycin could be achieved in approximately 85% (18).

Clofarabine a deoxynucleoside analogs is effective antileukemic drug not associated with the neurotoxicity observed with other analogs (19). In relapsed or refractory patients with AML response rate is about 50% (20). For newly diagnosed older patients CR rate with clofarabine in combination with cytarabine (the rational of combination is to modulate cytarabine triphosphate accumulation and decrease its toxicity) is achieved in 60% cases (21).

Genasense is another new agent known as antisense oligonucleotide which inhibits BCL-2 (22). BCL-2 as an apoptosis inhibitor protein can render leukemic cells resistant to induction of apoptosis. This antisense oligonucleotide combined with chemotherapy in phase II study could achieve CR rate in about 45% of patients with relapsed/refractory AML (23). At the moment the role of drug is investigating in induction and postremission therapy in phase III trial.

Agents that might be effective in AML are P-glycoprotein modulators. P-glycoprotein is a cellular membrane protein encoded by MDR1 gene. It serves as an efflux pump to extrude cytotoxic drugs from the cell (24). A high expression of P-glycoprotein is usually found in older patients with AML and in those with relapsed or refractory AML (25). This might explain refractoriness of these patients to standard chemotherapy. Several agents are able to overcome this refractoriness by blocking the efflux pump. MDR modu-

Table 1.

NEW AGENTS IN AML

Agent	Target	Class
Gemtuzmab ozogamomycin	CD33	Antibody/Immunoconjugates
Clofarabine	DNA	Deoxyadenosine analogs
Genasense	BCL-2	Apoptosis inhibitor
Zosuquidar, PSC833,	P-gp	MDR inhibitors
Tipifarnib	Lamins	FT inhibitors
Valproic acid, SAHA, depsipetide	HD AC	Histone deacetylase cytarabine(HD AC) inhibitors
Bevacizumab	VEGF	Antiangiogenic agents
PKC-412,CEP-701, MLN518,SU11248	FLT3 ITD	FLT3 inhibitors

Abbreviations: MDR – multidrug resistance, P-gp – P-glycoprotein, FT – farnesyltransferase, FLT3 ITD - fms-like tyrosine kinase 3 internal tandem duplication; SAHA, suberoylanilide hydroxamic acid; VEGF, vascular endothelial growth factor

lators such as cyclosporine and PSC-833 have no shown benefit in AML. More potent second generation modulators /*Zosuquidar* – formerly LY335979) is currently being investigated (26).

Farnesyltransferase inhibitors (FTI) also show activity in AML because FTI inhibitors interfere with RAS signaling farnelysation of RAS and its transfer to the plasma membrane (27). This is important step in signaling process. It is well known that mutation of RAS gene is associated with the development of myeloid leukemia (28). The overall response on oral agent FTI, Tipafarnib in newly diagnosed older patients with AML is 34% (29).

The remodeling and restoration of nuclear chromatin is another important mechanism of leukemogenesis. Histone acetyltransferase promote the remodeling of chromatin. The opposite effect and restoration of chromatin configuration is controlled by histone deacethylase (HDACs). By its inhibitors it is possible to induce the differentiation of leukemic precursor cells (30). Several new agents as *suberoylanilide hydroxamic acid /SAHA/, valproic acid, depsi-peptide and MS-275* acting as HDACs inhibitors are currently testing in phase II clinical trials (31, 32).

Increased microvessel density was documented in bone marrow biopsies from patients with AML. By adding vascular endothelial growth factor (VEGF) it is possible to stimulate growth and proliferation of leukemic cells (33). Growth stimulation by VEGF might be blocked by the use of receptor inhibitors of VEGF. Such activity has been demonstrated for small molecule inhibitor of phosphorilation of VEGF receptor SU5416. Another agent, anti-VEGF antibody, *Bevacizumab* was tested in phase II clinical trial. CR rate in patients receiving Bevacizumab after chemotherapy was achieved in about half of them (34).

In 30% of AML patients mutations of FLT3 receptor tyrosine kinase was described (35). This constitutive activation of FLT3 receptor tyrosine kinase is associated with poor prognosis of AML. Recently FLT3-selective targeted tyrosine kinase inhibitors have been developed. At the moment four agents with in vitro cytotoxicity of leukemia cells are undergoing investigation in clinical trials (36-38). Clinical responses are modest, and only transient reduction of blasts has been observed. Current clinical trials focus on the evaluation of FLT3 inhibitors in combination with chemotherapy.

ACUTE PROMYELOCYTIC LEUKEMIA (APL, AML-M3):

More than 90% of patients with APL have the balanced translocation t (15; 17) (q22; q11.12) involving the retinoic acid receptor-α (RAR-α) gene on chromosome 17 and PML gene on chromosome 15 (39). The new fusion oncogene governs the synthesis of PML/RAR-α fusion protein. The protein has reduced sensitivity to retinoic acid, preventing terminal differentiation of malignant promyelocytes. This defect can be overcome with the use of *trans-retinoic acid (ATRA).* ATRA therapy accelerates the terminal differentiation of malignant promyelocytes to mature neutrophils leading to apoptosis and CR without myelosuppression and bone marrow hypoplasia (40). Recent results of phase III trials proved a very high efficacy of ATRA and chemotherapy for remission induction, consolidation and maintenance therapy. The cure rate is about 70-75 %(41-42).

Arsenic trioxide (ATO) as ATRA, affects both apoptosis and partial differentiation in APL by interacting with the PML/RAR-α protein (43). ATO is also effective in patients who relapsed after ATRA treatment (44). *Gemtuzumab ozogamomycin* is recently shown to be very effective in APL (45), because the CD-33 antigen is strongly expressed in APL cells. Many of the clinical trials currently are investigating the combination of ATRA, ATO and Gemtuzumab ozogamomycin in remission induction, consolidation and maintenance therapy (46). The goal of such approach is to reduce or even omit the need for chemotherapy.

MYELODYSPLASTIC SYNDROME

Myelodisplatic syndrome is a clonal disorder of myeloid stem cells, resulting in alterations of differentiation and maturation of erythrocyte, platelet and granulocytic lineage. In MDS a frequent clonal, non-random chromosomal deletions (7q-, 5q-, 20q-, 6q- 11q- and 13q-) was re-

ported. These changes appear to inactivate tumor suppressor genes required for the normal development of myeloid cells (47). Tumor suppressors are very difficult to identify compare to the oncogenes activated by chromosomal translocations reported also in MDS. The deleted regions detected by cytogenetic methods are very large, containing many genes, thus making it hard to locate the critically affected gene or genes. Because of that therapy of MDS is still non-specific or better to say symptomatic. For the advanced stages of MDS with the signs of transformation to AML, treatment approach is similar as in AML. Targeted therapeutic advances in MDS will likely depend on a full comprehension of underlying molecular mechanisms, in particular the tumor suppressor genes lost through clonal, nonrandom chromosomal deletions, such as the 7q– and (del) 5q.

CHRONIC MYELOID LEUKEMIA

Philadelphia chromosome, a cytogenetic marker of CML, is reciprocal translocation of the c-ABL gene on chromosome 9q34 to the chromosome 22. The new BCR-ABL fusion oncogene on chromosome 22 encodes synthesis of Bcr-Abl fusion proteins. These proteins have the ability to promote leukemogenesis. The Bcr-Abl fusion protein contains the entire tyrosine kinase catalytic domain from c-Abl and has constitutively increased tyrosine kinase activity (48). With the understanding of a unique gene product and its activity many efforts have been made to develop compounds that could selectively inhibits aberrant tyrosine kinase. The compound which was known to bind to the ATP binding site of protein kinases were synthesized and screened for biological activity. One such compound termed STI-571, was found to be a potent inhibitor of the Bcr-Abl protein tyrosine kinase (49). This agent inhibited cellular proliferation and tumor formation by Bcr/Abl expressing cells without inducing apoptosis, and produced a 92 to 98 percent decrease in CML colony growth in vitro without inhibiting normal colony growth (50). This compound was renamed to imatinib mesylate (Gliveec) and currently is the first line therapy of Ph positive CML. The recommended initial starting dose of imatinib is 400 mg/day for patients in chronic phase and 600 mg/day for patients in accelerated phase or blast crisis (51). Doses less than 300 mg/day are considered subtherapeutic and should rarely be used. Responses to imatinib may occur at the hematologic, cytogenetic and molecular levels. The defining criteria for the various levels of response are summarized in table 2.

Table 2.

DEFINITIONS OF RESPONSE ON IMATINIB IN CML

Level of Response	Definition
Complete Hematologic response (CHR)	Normal full blood count and white cell differential count, no evidence of extramedullary disease
Minimal cytogenetic response	66% - 95% Ph-positive metaphases*
Minor cytogenetic response	36% - 65% Ph-positive metaphases*
Partial cytogenetic response	1% - 35% Ph-positive metaphases*
Complete cytogenetic response(CCR)	0% Ph-positive metaphases
Major cytogenetic response (MCR)	0% - 35% Ph-positive metaphases
Major molecular response (MMR)	= 3-log reduction of BCR-ABL mRNA
Complete molecular response (CMR)	Negativity by RT-PCR

* Based on the analysis of at least 20 metaphases

Those patients who achieve both complete cytogenetic response and >3 log reduction in the Q-PCR signal, 100 percent were alive and free of progression at 24 months (52).

A very small proportion of patients are resistant to treatment with imatinib. In addition, many patients with initial responses to imatinib ultimately relapse. This proportion for chronic phase is between 10 to 15% while in patients with blastic crisis it is estimated to be 80% (53) at two years. Drug resistance to imatinib is generally a consequence of reactivation of Bcr/Abl signaling, which can be due to bcr/abl overexpression, excretion of imatinib from the cell by transmembrane transporters, or, most commonly, by the development of single nucleotide mutations in bcr/abl which result in amino acid substitutions that change the conformation of the ATP binding site. In such situation imatinib no longer binds to mutant bcr/abl effectively. More than 40 such mutations have been described to date (54, 55). While some of these confer only modest increases in the resistance to imatinib,

others produce profound resistance in vitro and in vivo, which cannot be overcome by dose increases.

A number of new tyrosine kinase inhibitors have been synthesized specifically to target the altered bcr/abl proteins not responding any more to imatinib. Phase I/II trials have been completed with AMN107 (nilotinib) and BMS-354825 (dasatinib) (56, 57) in patients refractory to, or intolerant of imatinib. Both drugs are orally bioavailable, have favorable toxicity profiles, are >100-fold more potent than imatinib in vitro, and have produced hematologic and cytogenetic responses in patients whose CML was no longer responsive to imatinib.

Although both drugs are effective against most of the known bcr/abl mutations, some of the mutations, most notably a mutation known as T315I, are also resistant to high concentrations of these newer agents (56, 57). Exploratory clinical trials are being planned to assess whether these more potent inhibitors might be employed, either alone or in combination with imatinib or other agents, as initial therapy.

In vitro culture studies have indicated that CD34+ CML progenitor cells can remain viable in a quiescent state in the presence of imatinib and growth factors (58). Mathematical modeling and in vitro studies also suggest that imatinib is a potent inhibitor of the production of differentiated leukemic cells but does not deplete the pool of leukemic stem cells As experience with the newer tyrosine kinase inhibitors matures, it is likely that the initial use of chemotherapy in imatinib failures will decline. The clinical problem will then be whether to refer patients who have a complete cytogenetic response to these new agents to hematopoietic cell transplantation or whether to continue the agents in the hope that the responses will be durable. Currently, most patients treated with these drugs have been followed for less than one year, so that the durability of these responses is not yet known.

OTHER CHRONIC MYELOPROLIFERATIVE DISORDERS

In the WHO classification, three common and well characterized diseases are considered as myeloproliferative disorders. These include: Polycythaemia Vera (PV), agnogenic myeloid metaplasia (AMM also called idiopathic myelofibrosis) and essential thrombocythemia (ET). The molecular pathogenesis of these MPDs is poorly understood. In CML molecular pathogenesis was identified by the examination of recurrent chromosomal translocations. In other MPDs recurrent chromosomal translocations were not found. One of the basic hallmarks of MPDs is an abnormal response to cytokines. Numerous studies showed the existence of autocrine stimulation or a cytokine receptor defects as the causes of abnormal cytokine response (59, 60). These results suggest that the cytokine hypersensitivity was due to downstream signaling defects and that these MPDs are accompanied by a constitutive kinase activity. Cytoplasmatic tyrosine kinase Janus kinase 2 (JAK2), a gene found on the short arm of chromosome 9 (9p) could explain in part this hypersensitivity. A mutation of JAK2 gene leads to constitutive tyrosine phosphorilation activity that promotes hypersensitivity to cytokines and induces erythrocytosis in a mouse model (61).

A single gain-of-function point mutation (Val617Phe, V617F) in JAK2 has been identified in 65 to 97 percent of patients with PV, 23 to 57 percent of those with ET, and 43 to 57 percent of those with AMM (62, 63). This discovery of the *JAK2 V617F* mutation is a major advance in enhancing our understanding of both the molecular pathogenesis and the clinical aspects of PV and other MPDs. This observation opens new approach for researches and may have implications for the diagnosis and classification of MPDs. Research can now focus on MPDs that are negative for the V617F mutation, which might constitute a distinctive entity. Further work is also needed to determine how a single mutation in JAK2 gives rise to three different diseases. Moreover, the *JAK2 V617F* mutation offers a molecular target for new drug discovery.

SUMMARY AND FUTURE DIRECTIONS

A myriad of new agents are now available for the treatment of myeloproliferative disorders. They are currently using either alone or in combinations with each other or with conventional che-

motherapy. This approach has the potential to change the standard of care for patients with myeloproliferative disorders. Treatment combinations of several agents which target more than one gene mutation, signal transduction pathway or antigenic determinant are under investigation. This may be the most promising therapy in the future. Many of these agents are currently under investigation in cooperative group trials. As the molecular diversity of myeloproliferative disorders continues to be explored, it is expected that new molecular defects will be discovered in the near future. By increasing the type and number of new molecular alterations for targeted therapy in the near future one may expect more effective treatment modalities with better treatment outcome for myeloid malignancies.

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