REVIEW ARTICLE

Some New Findings on the Pathogenesis of Myeloproliferative Disorders and Possibilities for More Effective Treatment

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SUMMARY

Advances in molecular biology and genomics have enabled a more detailed view of the pathogenesis of myeloproliferative disorders, which are regarded as diseases of the hematopoietic stem cell. The author provides a brief overview of the genetic alterations leading to chronic myeloid leukemia, myelodysplastic syndrome and acute myeloid leukemia. In the second part, molecular targeted therapies that have been developed based on these insights are reviewed. These are, in particular, methods preventing increased proliferation, such as inhibition of tyrosinkinases (imatinib, dasatinib), inhibition of farnesyltransferase (tipifarnib), inhibition of angiogenesis (bevacizumab, vatalanib), induction of differentiation (hypomethylating agents) and induction of apoptosis (bortezomib). More detailed information is provided on some novel drugs which are currently in clinical trials.
Key words: leukemic hematopoiesis, myeloproliferative disorders, novel therapies. *Kl.*

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Advances in molecular biology in the last decade have improved our understanding of the pathogenesis of all forms of hematological malignancies. A logical consequence of such advances is to employ them in diagnostic methods, estimation of prognosis and implementation of new therapeutic algorithms. It is very difficult to summarize new findings in a short review. We shall try to highlight the most important of them, especially those concerning myeloproliferative diseases.

PATHOGENETIC MECHANISMS IN THE DEVELOPMENT OF MYELOPROLIFERATIVE DISEASES

Most myeloproliferative diseases are thought to be caused by disorders of the hematopoietic stem cell (13). In terms of structure, malignant hematopoiesis resembles normal hematopoiesis, from which it originated, together with the leukemic stem cell standing highest in the hierarchy. Leukemic stem cells originate from a malignant shift of normal hematopoietic stem cells due to a genetic mutation. It is thought at present that transformation of a normal cell into a leukemic one requires two mutations: mutations of two different types, in other words (6, 7, 14).

The first group of mutations includes ***a mutation of a gene for the receptor of the tyrosinkinase*** (most often due to chromosomal translocation). A prototype of this group of diseases is, for example, **chronic myeloid leukemia (CML).** This develops as a chromosomal translocation t(9:22) resulting in the transfer of a gene for the receptor of the tyrosinkinase ABL (Abelson Leukemia) into the region of BCR (Breakpoint Cluster Region). It results in the development of a chimeric gene producing a fusion protein *bcr-abl* with tyrosinkinase activity. While the receptor tyrosinkinase ABL is activated only when bound to a specific ligand, its translocated form BCR-ABL is aberrantly active and sends signals to a nucleus, resulting in increased proliferation of an autonomic (leukemic) clone. That clone then matures. The presence of a BCR-ABL translocation proves the fact that the malignant transformation took place at the level of a hematopoietic stem cell.

The other group of mutations essential for leukemogenesis comprises ***mutations of genes of hematopoietic transcription factors*** (point mutations, chromosomal deletions, translocations). If mutations of genes for the receptor tyrosinkinase lead to activation of appropriate tyrosinkinases, mutation of a gene for the hematopoietic transcription factor leads to their inhibition. This leads to a differentiation block resulting in the presence of a certain percentage of immature cells (leukemic blasts) in the bone marrow or in the peripheral blood, and subsequently to the development of an autonomic clone with dysplastic features and no proliferative activity. The prototype of this group of diseases, with mutations of hematopoietic transcription factors, are **myelodysplastic syndromes (MDS)**.

In cases when a ***combination of both types of mutations*** takes place, i.e. a mutation (activation) of a receptor tyrosinkinase and a mutation (inhibition) of a hematopoietic transcription factor, it leads to transformation to **acute myeloid leukemia (AML)** combining increased proliferative potential of leukemic cells with a differentiation block. The blood count therefore shows leukocytosis with the typical hiatus leukemicus, i.e. a presence of immature dysplastic (blastic) elements next to normal mature cells, the number of which gradually decreases due to expansion of the

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Tab. 1: Classification of acute leukemias based on the WHO classification (Blood 2002, 100, 2292-2302)

AML with genetic abnormalities
AML with a translocation t(8:21) (q22:q22), (AML1/ETO)
AML with abnormal eosinophilia inv(16) (p13:q22) or T(16:16)(P13:q22)
Acute promyelocytary leukemia t(15:17) (q22:q12), (PML/RAR-alfa)
AML with chromosomal abnormity 11q23
AML with multiline dysplasia
AML after preceding MDS
AML without preceding MDS more than 50 percent of dysplastic cells
AML and MDS after therapy
AML after therapy with alkylating drugs.
AML after therapy with topoisomerase II inhibitors
Other AMLs
AML with minimal differentiation
AML without maturation
Acute myelomonocytary leukemia
Acute monoblastic/monocytic leukemia
Acute erythroid leukemia
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma

leukemic clone. Other mutations also lead to malignant transformation of cells. These mutations lead not only to stimulation of proliferation and blockage of differentiation but also to inhibition of apoptosis and increased self-renewal of leukemic stem cells.

A very simplified diagram showing the mechanisms responsible for the malignant transformation of a hematopoietic stem cell and examples of genes causing the change in the biological characteristics of the cell can be found in Fig. 1.

The findings concerning the pathogenesis of leukemia have led to both its re-classification and the search for more effective modalities of treatment. The French-American--British classification (FAB) is still used for the classification of AML. It classifies leukemia based on the morphology of leukemic cells (2). A newly recommended WHO classification, shown in Table 1, takes into account the cytogenetic characteristics of individual forms of leukemia and enables differentiation of prognostically unfavorable forms that should be treated using different strategies (26).

CURRENT AND PERSPECTIVE TREATMENT MODALITIES

While the correction of particular abnormalities of ***genetic therapy*** is still subject to intensive research and not yet applicable in clinical practice, there are clinically approved methods which prevent the development of these abnormalities or assist in the correction of their consequences, i.e. they limit proliferation, angiogenesis or the metastatic potential or induce blocked differentiation or apoptosis (1). We will try to provide a short summary of these new therapeutic modalities.

Classical chemotherapy still is the basic therapeutic method for acute leukemias. Allogenic transplantation of hematopoietic stem cell may be used in indicated cases. It is considered to be the only curative method. In clinical practice, however, some new therapeutic modalities are being used, and a variety of new methods are being tested in clinical trials.

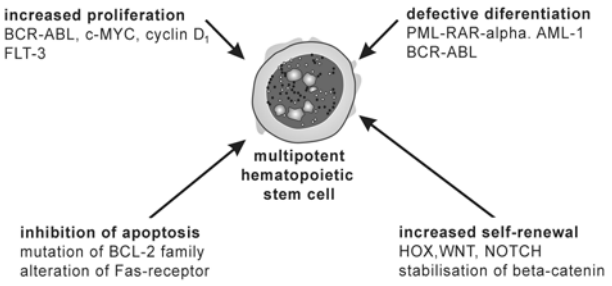

Fig. 1: A diagram of mechanisms responsible for the malignant transformation of a multipotent hematopoietic stem cell and examples of genes causing the change in biological behavior of a cell.

New preparations with cytotoxic effects

Homoharingtonine is a herbal alkaloid harvested from the needles and crust of a Chinese tree Cephalotoxux fortueni, which is used in treatment of CML. Its anti-neoplastic effect is interpreted as inhibition of the BCR-ABL gene expression; in addition, it inhibits protein synthesis and degradation of polyribosomes (4). Recommended doses are 3–6 mg/day administered in saline in a five-day cycle. Side effects include vasodilatation, hypotension and myelosupression.

5-azacytidine (Vidaza) was synthesized in the Czech Republic in the 1960s and later isolated in the U.S.A. from the Streptoverticilium ladacamus cultures as an antibiotic ladacadamycine. Its widespread use in hematooncology has occurred only in recent years. Its characteristics, apart from the cytostatic effect, include the effect of hypomethylation. Increased methylation of some regions of DNA significantly increases the proliferative potential of cells and causes inhibition of recessive oncogenes (tumor-suppressing genes). Re-expression of these genes enables demethylation, which can induce even blocked differentiation of cells. Therefore this preparation has also been employed in MDS, applied in a recommended dose of 75mg/m^2/day in a seven-day cycle once a month or 15 mg/m^2/day in a ten-day cycle. It is also being tested in the treatment of leukemia or a blastic shift of CML, where higher doses (50-100 mg/m^2 for 5 days) induce up to 14 per cent of cytogenetic remissions (16).

Decitabine (Dacogen, Mylosar), 5-aza-2'deoxyazacytidine is a substance with a similar hypomethylating effect. Its activation (phosphorylation) is catalyzed by deoxycytidinkinase, which is found in high concentrations in the lymphatic tissue. It is being tested in the treatment of acute lymphoblastic leukemias and MDS. The dose is 75 mg/m^2/day for 1 week.

Tretinoin –Vesanoid (all-trans-retinoid acid-ATRA) is a natural metabolite of retinol (vitamin A) with strong differentiating effects on malignant promyelocytes. The effect results from the suppression of the PML/RAR-alpha fusion gene, which originates from a translocation of chromosomes 15 and 11. A chimeric protein, a product of the mentioned gene, inhibits transcription of differentiating genes and thus enables increased proliferation of leukemic cells. Tretinoin is applied *per os* in a dose of 45 mg/m^2/day twice a day until remission is achieved. The so-called retinoid syndrome, a possible complication of the therapy, is manifested by fluid retention, exsudations, fever and leukocytosis.

Anagrelid (Thromboreductine, Agryline) is an imidazolchinoline derivate. It decreases maturation of megakaryocytes and limits synthesis of trombocytes. It also has a strong anti-aggregation effect. It is an effective drug in the therapy of trombocytemia. With a dose of 0.5 mg *per os* QID (maximum of 10 mg/day) it is possible to

achieve reduction in the number of thrombocytes as early as the first two weeks.

Monoclonal antibodies (MA)

MA are predominantly used in the therapy of lymphoproliferative diseases. Their application potential is summarized elsewhere (19). An antibody against the CD33 antigene, which is expressed in leukemic blasts, is already used in clinical practice in the therapy of AML. The effectivity of this antibody is reinforced by its conjugation with a cytotoxic antibiotic called clichemycine, with the generic name **gemtuzumab ozagamycine** (Mylotarg). This has been effective in the treatment of an AML relapse, where it induces up to 30 percent of remissions. It is usually applied in a dose of 9 mg/m^2 in a solution intravenously; the tolerance is good, and the most common side effects include fever, nausea and myelosuppression (11).

Monoclonal antibodies may also be used in the inhibition of extracellular domains of membrane receptors. An example of this is a humanized monoclonal antibody against the vascular endothelial factor VEGF **bevacizumab** (Avastine); as has been shown, excessive angiogenesis may inversely influence development of acute leukemia. The effectivity of inhibition of angiogenesis is explained by increased expression of KDR and c-KIT receptors on leukemic cells. c-KIT receptors are a binding place for VEGF (18). Angiogenesis inhibition using substances inhibiting the intracellular domains for VEGF receptors is at an advanced stage of clinical research (see below).

Transduction cascade inhibition

Transduction cascade inhibition represents a type of so-called targeted therapy, i.e. influence of intracellular pathways leading to the shift of a normal cellular phenotype into a malignant one. Complicated intracellular pathways of signaling can be specifically affected at various levels.

(1) Inhibition of tyrosinkinases is the most studied area of interest. Proteinkinases comprise a large family of kinases, some of them activated by a bond to a specific ligand on a membrane receptor (21), while others are activated via intracellular pathways.

Receptor tyrosinkinases. This category includes tyrosinkinases for the EGF receptors (epidermal growth factor), PDGF (platelet derived growth factor), FGF (fibroblast growth factor), HGF (hepatocyte growth factor) and VEGF. Inhibitors of EGF are already being tested in the therapy of various solid tumors (erlotinib – Tarceva, gefitinib – Iressa). **Semaxanib (SU5416)** could be useful in the treatment of hematological malignancies. It is an indolin derivate that inhibits a tyrosinkinase receptor for VEGF and thus limits angiogenesis (5). After receiving promising results from the 1st and 2nd stage of clinical trials, the research has proceeded to the 3rd stage of a clinical trial in various malignancies; however, the expected effectivity failed to materialise, and consequently the study was aborted. On the other hand, there has been good experience using an anilinoftlalazine derivate with the generic name **vatalanib (PT 787/ZK 33584).** This preparation inhibits not only the intracellular domains of the VEGF family of receptors but also a domain on a PDGF receptor, c-KIT and c-FMS. It is applied in a dose of 500 mg daily *per os*. A similar effect has been achieved using a newly introduced preparation **sorafenib.** A lot of effort has been invested in preparing an inhibitor to a receptor kinase FLT3 because receptors for FLT3 are overexpressed on leukemic blasts (8). Several inhibitors of this receptor are currently being tested on mice models (22).

Non-receptor (cytoplastic) tyrosinkinases play an important role in signal transduction. They are activated via various intracellular processes (24). Almost 30 non-receptor kinases have been described; the most important of the tyrosinkinases are SRC, ABL and JAK. By inhibiting these tyrosinkinases, blockade of a malignant cell transformation can be expected. **Imatinib mesylate** (Glivec) is a clinically verified inhibitor of the BCR-ABL tyrosinkinase, so far the most effective drug for the CML. It induced hematological remission in 90 percent of cases; in 60–70 % of patients it induced cytogenetic remission (20). It inhibits the tyrosinkinase of a pathological fusion gene BCR-ABL. The aberrant proteinkinase activity of this gene is responsible for the intensive proliferation of myeloid precursors. Imatinib also blocks other tyrosinkinases. It is an inhibition of a c-KIT, the increased expression of which is observed in cells of the gastrointestinal stromal tumor (GIST), and also inhibition of the FIP1L1-PDGFR gene activity, that is responsible for the hypereosinophilic syndrome and eosinophilic leukemia (3). The effectivity of imatinib in these diseases has already been clinically verified. The inhibitory effect on the tyrosinkinase for the PDGF receptor, a mitogene of the connective tissue, shows the possibility of also using imatinib in the treatment of the myelofibrosis syndrome and some other oncological diseases with increased expression of the PDGFR (27). **Dasatinib** is a dual inhibitor of the SRC and ABL kinases. It is not structurally similar to imatinib, but it is 100–300 times more effective, even in cases resistant to imatinib. Dosing is not stable. In clinical studies, 25–180 mg/day in two doses have been applied. Another tested inhibitor with similar properties is the **AMN-107** (28).

(2) Inhibition of the farnesyltransferase. RAS proteins play a significant role in signal transduction. They are, to a certain degree, responsible for mitogenic stimulation. It has been proven that mutation of the RAS oncogene is found in more than one third of malignancies. The K-RAS in non-small cell lung carcinoma, in pancreatic carcinoma and in a colorectal carcinoma, the H-RAS in carcinomas of the urinary bladder, kidneys and thyroid gland, and the N-RAS in hematological malignancies and melanoma. The RAS cascade activates nuclear oncogenes (FOS, JUN, and others) regulating cellular proliferation. The RAS activation is dependent on reaction of the protein with a lipid (farnesylation), which is an enzyme activated reaction using farnesyltransferase. Inhibition of farnesyltransferase blocks the function of the ras protein and thus the main transduction cascade pathway. More than 20 different inhibitors have been tested; so far only two have been applied in clinical practice (17). These are a preparation with a code sign **BMS 214662**, and also **lonafarnib** (Sarasar) which is clinically tested in selected solid tumors and **tipifarnib** (Zarnestra) which seems to be effective in refractory and relapsing AML (9). It has been proven effective even in a blastic shift in CML in a dose of 300 mg *per* os twice a day for 2–3 weeks (15).

(3) Inhibition of proteasome and induction of apoptosis. Proteasome is a multicatalytic enzymatic complex. It plays a key-role in protein management, regulating cell cycle and the process of apoptosis. Disturbing the degradation of these proteins may lead to slowing or stopping the cell cycle of fast-proliferating cells (10). In addition, proteasome significantly interacts with the regulation of apoptosis via the transcription factor – nuclear factor kappa B (NFkB). This factor activates gene transcription of growth factors (IL-6, angiogenic and antiapoptotic factors). Inhibition of the proteasome can therefore not only suppress proliferation but also induce apoptosis by inhibiting NFkB (23). The only proteasome inhibitor used in clinical practice so far is a derivate of a boronic acid bortesomib (Velcade). It has become an important part of the spectrum of drugs used in the treatment of multiple myeloma; however, it is also being tested in other hematological malignancies (12, 25). It is applied in a dose of 1–1.3 mg/m^2 twice a week for two

weeks followed by a 10-day break. Most common side effects include anorexia, nausea, fatigue, diarrhoea, fever, sensory neuropathy and thrombocytopenia.

Proapoptotic effects were demonstrated in some other substances, including **arsentrioxide** (Trisenox), which has an antiproliferative effect by increasing the expression of cyclin-dependent kinase (cdk) inhibitors. Its proapoptotic effect is explained by activation of cystein proteases (caspases), inhibition of NFkB and induction of the bax protein expression. It inhibits the effect of the bcl-2 protein, a strong inhibitor of apoptosis. These characteristics are used in the treatment of promyelocytic leukemia, and it is also being tested in the treatment of the MDS. **Thalidomid** (Thalomid, Celgen) is a very effective inductor of apoptosis, even though its actions are complex. It inhibits TNFα, increases expression of adhesive molecules and it also has strong antiangiogenic properties. It has become a part of treatment modalities in multiple myeloma (in a dose of 200 mg/day with a maximum daily dose of 800 mg); in myeloproliferative disorders it is used in the treatment of the MDS even though its more effective derivate **lenalidomid** (Revlimid, Actimid) is usually preferred.

There have been many substances in experimental research that could possibly influence intracellular processes. These include inhibitors of proteinkinases M-TOR (mammalian targed of rapamycine), which is a proteinkinase participating in translation and transcription processes; inhibitors of histon deacetylase and also some extracts of natural substances. These includeberbamine, an extract from a sea celenterate *Berberis amurensis*, effective in the treatment of CML, and aplidine, an extract from an herb *Aplidium albicans,* effective in the treatment of acute leukemias.

CONCLUSION

As emphasized in the introduction, the origin and the course of myeloproliferative disorders is implication of genetic changes. It is evident that a breakthrough may be brought about by genetic therapy. Even though various methods of genetic therapy (especially synthesis of anti-oncogenic vaccines) are being submitted to intensive research, there is still a long way ahead of us before it is possible to use these methods in clinical practice. It is therefore necessary to make the most of the therapeutic modalities currently available. A summary of these is provided in

Tab. 2: Summary of newly introduced or tested preparations in treatment of myeloproliferative diseases

AML	APL	MDS	CML
gemtuzumab	vesanoid	5-azacytidine	imatinib
tipifarnib	arsentrioxide	decitabine	dasatinib
bevacizumab		thalidomid	AMN 107
vatalanib		lenalidomid	homoharingtonine
5-azacytidine?			berbamine

AML = acute myeloid leukemia, APL = acute promyelocytary leukemia
MDS = myelodysplastic syndrome, CML = chronic myeloid leukemia

Table 2.
List of used abbreviations

AML	- acute myeloid leukemia
ABL	- abelson leukemia
BCR	- breakpoint cluster region
BCR-ABL	- pathological fusion gene
cdk	- cyclin-dependent kinase
CML	- chronic myeloid leukemia
EGF	- epidermal growth factor
FAB	- the French-American- British classification
FGF	- fibroblast growth factor
GIST	- gastrointestinal stromal tumor
HGF	- hepatocyte growth factor
JAK	- Janus kinase
MDS	- myelodysplastic syndrome
MA	- monoclonal antibodies
NFκB	- nuclear factor kappa B
PML/RAR-alfa	- fusion gene
PDGF	- platelet derived growth factor
PDGFR	- receptor for PDGF
VEGF	- vascular endothelial growth factor
WHO	- World Health Organization

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