Characteristics of Two Mouse *bcr-abl*-Transformed Cell Lines. II. Pathological Lesions Induced in Mice

(*bcr-abl*-transformed cells / implantation / mice / pathological lesions)

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Abstract. Groups of six BALB/c mice each were intravenously inoculated with lethal doses of Ba-P210 (B210) or 12B1 cells and examined by autopsy, histology, special staining methods, enzyme histochemistry and immunohistochemistry. Clinical symptoms related to neoplasia consisted of a poor nutritional state, anaemia, mild to moderate dehydration and apathy. Paresis was apparent in three mice inoculated with 12B1 cells. Necropsy revealed splenomegaly in all animals. Sporadic haemorrhages in the lungs and enlargement of some lymph nodes were seen in some of the animals. Histological examination showed neoplastic cells in the spleen, in the bone marrow of the sternum, in the lung interstitium and in sinusoids of the liver in all mice. In six of nine brains examined, mild to moderate infiltration by neoplastic cells was observed. In all but two mice mild infiltration of the kidneys was found. The enlargement of lymph nodes was caused by an accumulation of neoplastic cells. The paresis was due to neoplastic infiltration of the vertebra, epidural space and spinal roots. Staining with Sudan black revealed cytoplasmic granules in neoplastic cells; however, the peroxidase reaction was negative. Numerous neoplastic cells disseminated in the red pulp of the spleen were reactive with CD3, CD79β, CD11b and with neutrophil antibodies. We classified the disease induced by both of the cell lines as acute myeloid undifferentiated leukaemia (AML MO).

Chronic myeloid leukaemia (CML) is associated with a chromosome abnormality known as the Philadelphia chromosome (Ph+). The Ph+ is a reciprocal translocation between chromosome 9 and chromosome 22. A portion of the protoncogene *abl* is tranferred from chromosome 9 to chromosome 22, where it fuses with the *bcr* (*breakpoint cluster region*) gene. The resulting fusion gene is denoted *bcr-abl*. The product of this gene, the chimeric Bcr-Abl protein, exhibits a high tyrosine kinase activity, significantly exceeding that exhibited by the product of the c-*abl* gene (Konopka and Witte, 1985; Daley et al., 1990; Jacobs et al., 2002). It is generally believed that this is the key event in the causation of CML.

Depending on the breaking point in the *bcr* gene, the product of the *bcr-abl* oncogene exists in three different forms denoted $p190^{bcr\text{-}abl}$, $p210^{bcr\text{-}abl}$ and $p230^{bcr\text{-}abl}$. These three proteins have different tyrosine kinase activity and different *in vitro* transformation characteristics. All of the three respective oncogenes are capable to transform both myeloid (32Dcl3) and lymphoid (Ba/F3) cell lines. The proliferation activity of these cells differs and correlates directly with their tyrosine kinase activity (Li et al., 1999). In a murine bone marrow transduction/transplantation model, the three forms of Bcr-Abl have been equally potent in the induction of a CML-like myeloproliferation syndrome. Analysis of proviral integration has shown this CML-like disease to be polyclonal and to also involve B-lymphoid lineages, thus implicating that the primitive multipotent stem cell is the target cell. Secondary transplantation of bone marrow induced a CML-like disease or B-lymphoid acute leukaemia in recipients (Li et al., 1999). Pear et al. (1998) induced a CML-like disease in mice by implanting bone marrow cells transduced by Bcr-Abl-encoding retroviral vectors. Three to five weeks after the bone marrow transplantation the disease manifested itself by cachexia, decreased motility and poor grooming. Peripheral white blood cell counts were elevated and the blood cell population was largely composed of polymorphonuclear neutrophils and metamyelocytes. Blasts were rare. Spleens were enlarged, firm and red. Histology of the spleens revealed marked expansion of red pulp and marked extramedullary haemopoiesis (EMH). Foci of necrosis were observed in some cases. Bone marrow was hypercellular and EMH was also detected in the liver. The studies performed in mice have recently been reviewed by Van Etten (2001) and Ilaria

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Abbreviations: CML – chronic myeloid leukaemia, EMH – extramedullary haemopoiesis, HE – haematoxylin and eosin, IPOX – immunoperoxidase method, i.v. – intravenous, PALS – periarteriolar lymphocytic sheaths.

(2004).

In a previous paper (Sobotková et al., 2005) we described the basic properties of two mouse *bcr-abl*-transformed cell lines, viz. BA-p210 (B210) (Daley and Baltimore, 1988) and 12B1 (McLaughlin et al., 1987). It was shown that they differ morphologically, in their sensitivity to imatinib mesylate, their expression of MHC class I molecules and their oncogenicity. The 12B1 cells were more than 100 times more oncogenic than B210 cells after intravenous (i.v.) administration and, in addition, were oncogenic after subcutaneous (s.c.) inoculation. In the present report we describe pathological findings in mice inoculated with either B210 or 12B1 cells.

Material and Methods

Cell lines

Two BALB/c mouse cell lines transformed by the *bcr-abl* gene (b3a2) and expressing the $p210^{bcr\text{-}abl}$ protein were used. Ba-P210 (B210) cells were kindly provided by G.Q. Daley (Whitehead Institute of Biochemical Research, Cambridge, MA). They had been derived from interleukin 3 (IL-3)-dependent BaF/3 cells (Daley and Baltimore, 1988). Their transformation by the *bcr-abl* gene carried out by a retroviral vector made them IL-3 independent. 12B1 cells (McLaughlin et al.,1987) were obtained through the courtesy of E. Katsanis (University of Arizona, Tuscon, AZ). They had been derived by transformation of primary bone marrow cells with a retrovirus-derived vector carrying the *bcr-abl* fusion gene. Either cells were passaged in RPMI-1640 medium (Sigma-Aldrich Corp., St. Louis, MO) supplemented with 10% FCS (PAA Labs., Linz, Austria), 2 mmol/l glutamine and antibiotics. In the case of 12B1 the medium was further enriched by 1 mmol/l sodium pyruvate and 50 μmol/l 2-mercaptoethanol.

Animals

In the present experiments BALB/c mice of SPF quality, purchased from Charles River, Sulzfeld, Germany, were used. At the start of the experiments the mice were 5–8 weeks old. The animals were housed in Macrolon type 3 plastic cages under standard, conventional conditions without regulation of the dark-light cycle. They were fed complete diet (pellets) *ad libitum* and given drinking water in glass bottles. All experimental procedures were carried out in accordance with the Guidelines for Animal Experimentation valid in the Czech Republic. Animals were inoculated i.v. with freshly harvested cells that had been washed with PBS. Counts of 5×10^5 or 10^6 B210 cells and 5×10^3 or 10^4 12B1 cells were administered.

Gross pathology

Mice were killed by cervical dislocation and necropsy was performed in the usual way. For histopathological examination, samples of the following organs were collected: brain, heart, lung, thymus, spleen, lymph nodes, sternum, liver, stomach, intestine, kidney, gonads, uterus or accessory genital glands and all organs with macroscopically apparent pathological changes.

Histopathology

Samples for histopathological examination were fixed in 10% buffered formalin. Some of them were also fixed in Davidson's solution. After fixation, samples were processed by the common paraffin method and 4 μm thick histological slices were stained with haematoxylin and eosin. Some slides were also stained after Giemsa.

Cytology

For cytological examination transversal sections of the spleen and liver from six mice inoculated with either B210 or 12B1 cells were imprinted on microscopic slides. Imprints were stained after Giemsa.

Sudan black C staining and enzyme histochemistry

For enzyme histochemistry and for immunohistochemistry, samples of the spleen were frozen and 4–5 μm thick cryocut sections were prepared from the samples by means of Cryocut E (Reichert-Jung, Nussloch, Germany). The sections, attached to microscopic slides, were fixed in formaldehyde vapour for 10 min.

Sudan black C solution was prepared by routine procedure, sections were stained for 10 min, differentiated in 70% ethanol and counterstained with Nuclear Fast Red (Fluka Chemie GmbH, Buchs, Switzerland).

Peroxidase was examined using a DAB Chromogen Kit (Immunotech, Marseille, France).

Non-specific esterase was detected by the method of Gomori as modified by Lojda (1970), using 10 mg α-naphtyl acetate (Sigma Chemical Co., St. Louis, MO) dissolved in 1 ml acetone p.a. and 50 mg Fast Blue BB salt (Lachema Brno, Czech Republic) dissolved in 50 ml 0.1 M phosphate buffer, pH 7.4. Slides were incubated at room temperature for 15 min.

Naphthol AS-D chloroacetate esterase activity was examined after Lojda (1970) by means of medium containing 10 mg of naphtol AS-D chloroacetate (Lachema Brno) dissolved in 0.5 ml acetone p.a. and 50 mg Fast Blue BB salt (Lachema Brno) dissolved in 50 ml 0.1 M phosphate buffer, pH 6.5. Slides were incubated at room temperature for 30 min and counterstained with Nuclear Fast Red (Fluka Chemie GmbH, Buchs, Switzerland).

Immunohistochemistry

CD3 was visualized with rat anti-human antibody, clone CD3-12, diluted 1 : 100; CD11b was detected with rat anti-mouse antibody, clone 5C6, diluted 1 : 100; CD79β was detected by hamster anti-mouse monoclonal

antibody (HM79-11) diluted 1 : 100, and neutrophils were detected with rat anti-mouse antibody (clone 7/4), also diluted 1 : 100. All of the antibodies were purchased from Serotec Ltd., Oxford, UK.

Immunohistochemical reactions were performed as follows. After the usual deparaffinization, cellular antigens were unmasked by boiling slides in 10 mM citrate buffer, pH 6.0, for 10 min. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide for 15 min. Non-specific binding was eliminated by a protein blocking agent (Immunotech) at 5 min exposure. Slides treated with primary antibodies were incubated in a humid chamber at room temperature for 60 min. The binding of biotinylated secondary antibody (Immunotech) and of the streptavidin-peroxidase reagent (Immunotech) followed, either for 10 min. In the CDβ reaction, rabbit anti-hamster IgG antibody labelled with peroxidase (Harlam Sera Lab. Ltd., Crawley Down, UK) was used as secondary antibody. Reactions were visualized using the DAB Chromogen Kit (Immunotech). Slides were counterstained with haematoxylin, dehydrated and mounted in Canada balsam.

Results

Clinical symptoms and gross pathology

Clinical symptoms and gross pathology are shown in Tables 1 and 2. They were essentially similar in mice inoculated with either B210 or 12B1 cells. Intravenous administration of 5×10^5 or 10^6 B210 cells was followed by clinically symptomless period of about 17 days. The disease then quickly developed in the majority of the animals and they usually died in 4 to 6 days. Paresis of pelvic limbs was not seen in any of the six B210-inoculated mice. Animals inoculated with 5×10^3 or 10^4 12B1 cells usually fell ill earlier, roughly after 12 days. The course of the disease was then similar to that in mice treated with B210 cells, but paresis was diagnosed in three cases.

Histopathology

Cytological characteristics were better preserved in samples fixed in Davidson's solution than in those fixed in formalin (Figs. 1, 2).

Histopathological lesions in the brain consisted in segmental infiltration of the meninges by neoplastic cells. In mice suffering from paresis, neoplastic cells infiltrated the epidural space of the lumbar spine and spread along spinal roots out of the vertebral canal. There was severe osteolysis of the vertebrae, caused by neoplastic cells originating from vertebral bone marrow (Fig. 3). 12B1 cells apparently possessed a higher tropism for the meninges than B210 cells and their infiltrates compressed both the spinal cord and spinal roots. Alterations in the heart, when present, were represented by small aggregates of neoplastic cells located in the subendocardial connective tissue and in the myocardium. With one exception, the presence of neoplastic cells was combined with numerous foci of calcification located in subepicardial connective tissue in the right ventricle. Neoplastic cells infiltrated the pulmonary interstitium in all mice. In some cases the infiltration was mild; however, in the majority it was quite dense, causing dystelectasis of the lung (Fig. 4). Haemorrhages present in the alveoli and in the interstitium were of various size and were observed both macroscopically and microscopically.

Affection of the spleen was the most conspicuous lesion in all mice. There was marked splenomegaly caused by EHP and by infiltration of neoplastic cells that effaced the original structure (Fig. 5). Reliable differentiation between developmental forms of myelopoiesis and neoplastic myeloid cells was difficult in some instances.

In some lymph nodes an accumulation of myeloid cells in the subcapsular sinuses was found. In mice Nos. 1, 2, 4, 6, 8 and 9 the neoplastic cells occupied, to a different extent, both cortex and medulla. Only in one

Table 1. Summary of clinical symptoms

Mice/Sex	Cells	Days	Nutr. state	Anaemia	Dehydration	Psychic activity	Paresis	Other
1 / F	B210	21	poor	mild	mild	apathy	no	no
2 / F	B210	21	poor	mild	mild	apathy	no	no
3 / F	B210	21	poor	mild	mild	apathy	no	no
4 / M	B210	17	poor	mild	no	apathy	no	conjunctivitis
5 / M	B210	17	poor	mild	mild	apathy	no	conjunctivitis
6 / M	B210	17	poor	mild	mild	apathy	no	no
7 / F	12B1	22	poor	moderate	moderate	apathy	yes	no
8 / M	12B1	17	poor	mild	mild	apathy	yes	susp. lymphadenopathy
9 / M	12B1	28	poor	moderate	moderate	apathy	yes	no
10 / M	12B1	12	good	no	no	active	no	no
11 / M	12B1	12	good	no	no	active	no	skin biting wounds
12 / M	12B1	12	good	no	no	active	no	skin biting wounds

Table 2. Summary of gross pathology

Anim./Sex	Cells	Days	Spleen size	Other findings
1 / F	B210	21	NR	NR
2 / F	B210	21	NR	NR
3 / F	B210	21	NR	NR
4 / M	B210	17	38 × 13 × 5 mm	Sporadic haemorrhages Ø 1 mm in the lung, subepicardial white strips in the right heart
5 / M	B210	17	25 × 11 × 4 mm	Sporadic haemorrhages Ø 1 mm in the lung, subepicardial white strips in the right heart
6 / M	B210	17	35 × 14 × 4 mm	Sporadic haemorrhages Ø 1 mm in the lung, subepicardial white strips in the right heart
7 / F	12B1	22	25 × 7 × 4 mm	No macroscopically apparent changes
8 / M	12B1	17	30 × 10 × 3 mm	↑ inguinal and mesenteric lymph nodes, sporadic haemorrhages Ø 1 mm in the lung, subepicardial white strips in the right heart
9 / M	12B1	28	25 × 8 × 3 mm	↓ thymus, ↑ paraaortal lymph nodes, one haemorrhage Ø l mm in the lung
10 / M	12B1	12	27 × 8 × 4 mm	Sporadic haemorrhages Ø 1 mm in the lung
11 / M	12B1	12	25 × 8 × 4 mm	Multiple small haemorrhages in subcutaneous tissue due to biting, no macroscopically apparent changes in internal organs
12 / M	12B1	12	28 × 8 × 4 mm	Multiple small haemorrhages in subcutaneous tissue due to biting, no macroscopically apparent changes in internal organs

NR – not recorded

mouse a neoplastic nodule was found in the thymus, very probably having spread there from the adjacent connective tissue.

Liver sinusoids were infiltrated by neoplastic cells in all mice. In some animals these cells were only sporadic, in others the infiltration was moderate or considerable and in these cases angioinvasion was apparent. In part of the portal fields, perivascular sheaths of myeloid cells had been developed (Figs. 6, 7). In addition to the presence of neoplastic cells, EHP of different intensity was observed in the liver parenchyma.

The salivary glands, the pancreas, stomach and the intestine were devoid of neoplastic cells in all mice in which these organs had been examined. In the kidneys, myeloid cells were only found in the glomerular capillaries. Except for one mouse (No. 3) neoplastic cells were not found in the gonads, the uterus or the accessory sex glands.

The bone marrow of the sternum was completely or almost completely infiltrated by neoplastic myeloid cells in all mice. The same was seen in histologically examined vertebrae and femora. Both in the sternum and the vertebrae neoplastic cells proliferated through the bone tissue into the surrounding muscles and connective tissue and infiltrated them to a different extent.

Cytology

Neoplastic cells were round and of different size (anisocytosis). Large cells of a diameter 3 to 5 times exceeding the size of the erythrocyte, containing a moderate to large amount of cytoplasm, predominated. The majority of the cells displayed deeply blue cytoplasm, in a portion of the cells it was eosinophilic. No cytoplasmic granules were seen. Sporadic small vacuoles were visible in the cytoplasm in some cells. Nuclei were predominantly round and oval. Bean-shaped, indented, or irregular nuclei were also present. Large nucleoli were seen in many cells, in some of them more than one per nucleus. Mitotic figures were relatively frequent, some were atypical (Figs. 8, 9). Binuclear cells were observed only sporadically. Chromatin was fine, dense, deeply basophilic. Besides neoplastic cells, also different developmental stages of erythro-, granulo- and thrombocytopoiesis were seen in spleen imprints.

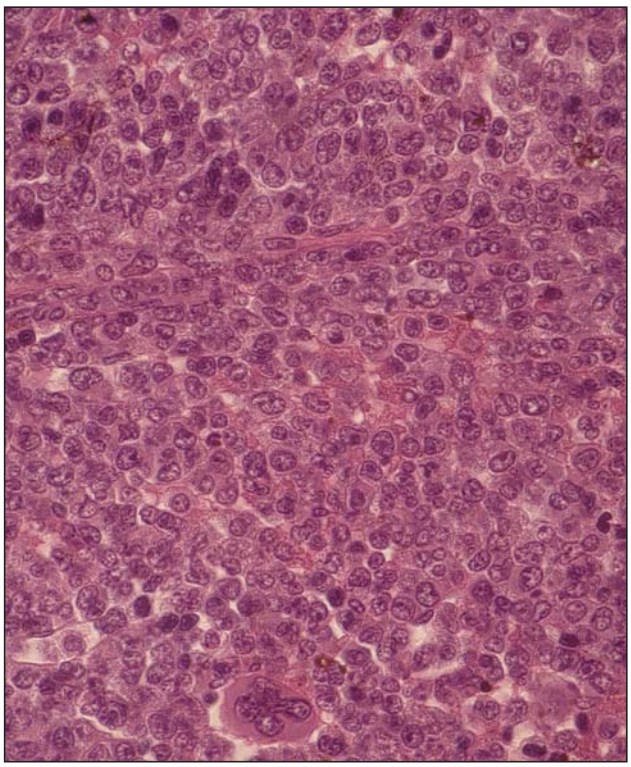

Fig. 1. Sample of a spleen fixed in Davidson's solution. B 210, 17 days. Staining with HE, × 150.

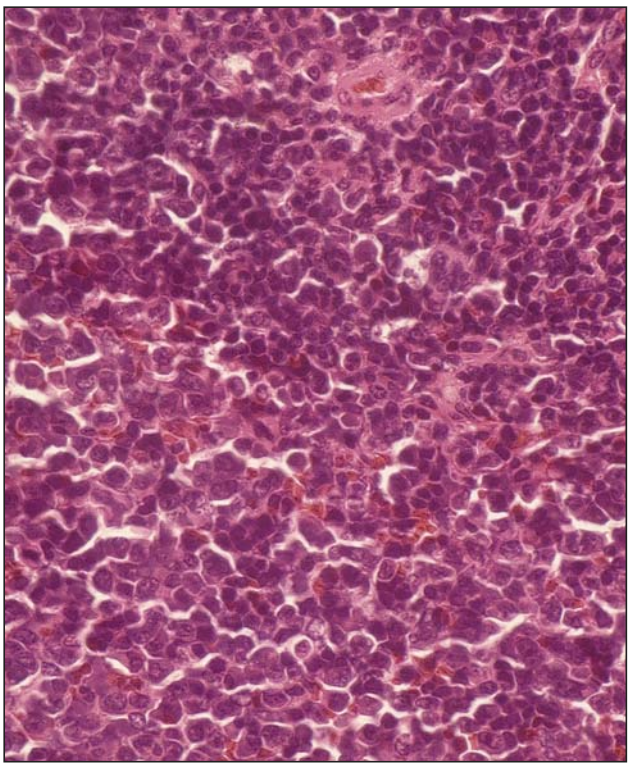

Fig. 2. Sample of the same spleen as in Fig. 1 fixed in 10% buffered formalin. B 210, 17 days. Staining with HE, × 150.

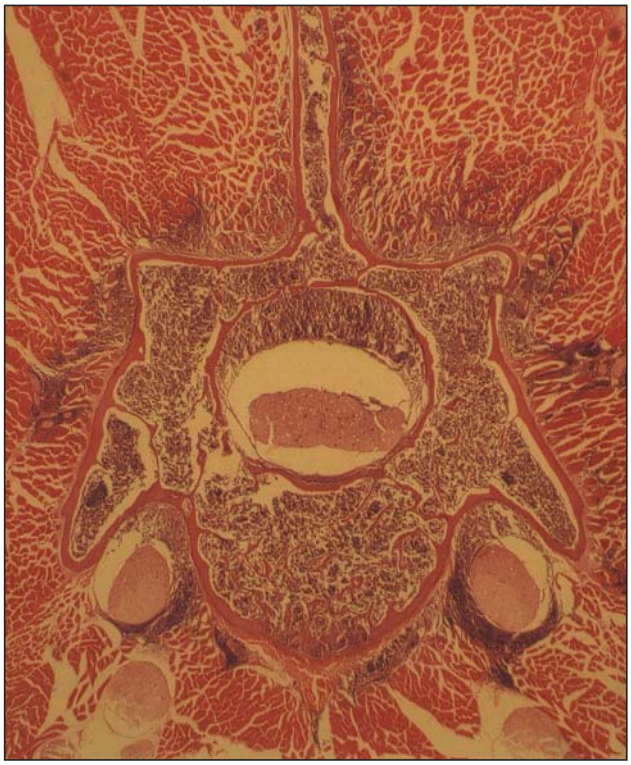

Fig. 3. Transversal section of a vertebra infiltrated by neoplastic myeloid cells. Besides of bone marrow there is marked infiltration in the bone tissue of the vertebra, in the epidural space, and in muscle tissue. Neoplastic cells spread along spinal roots. 12B1, 17 days. Staining with HE, × 3.

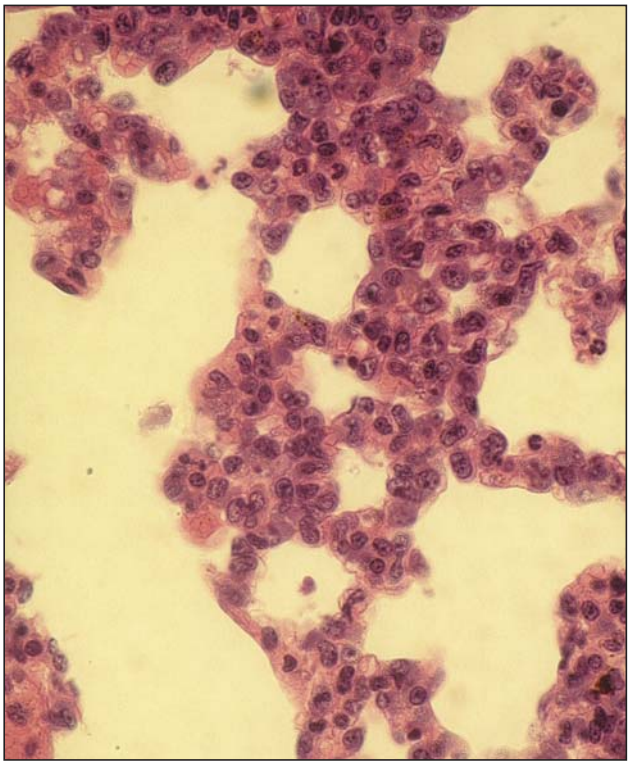

Fig. 4. Infiltration of the pulmonary interstitium by neoplastic myeloid cells. B210, 17 days Staining with HE, × 150.

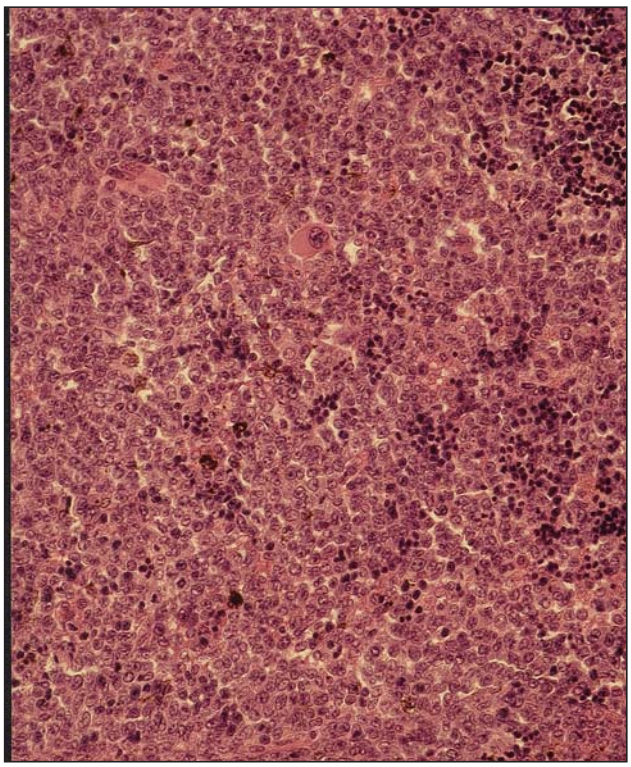

Fig. 5. Infiltration of the spleen by neoplastic myeloid cells. B210, 17 days. Staining with HE, × 80.

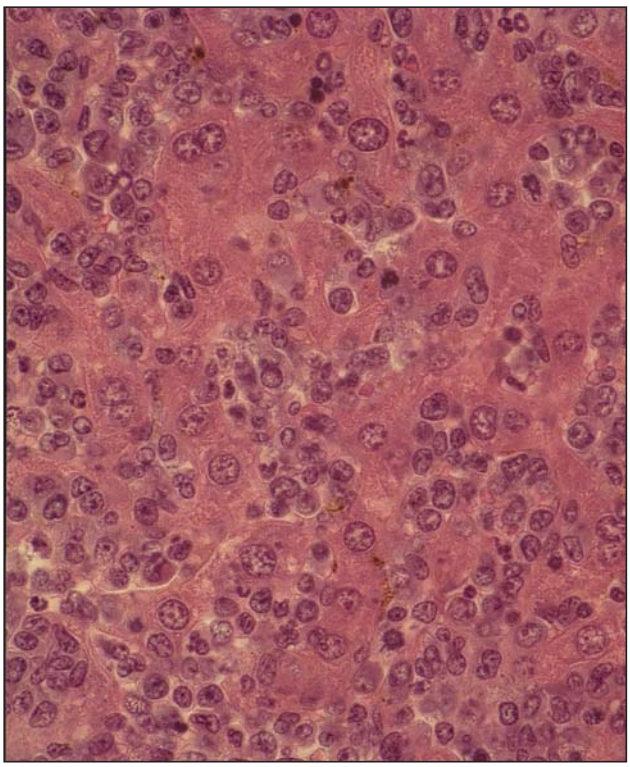

Fig. 6. Infiltration of liver sinusoids by neoplastic myeloid cells. B210, 17 days. Staining with HE, × 150.

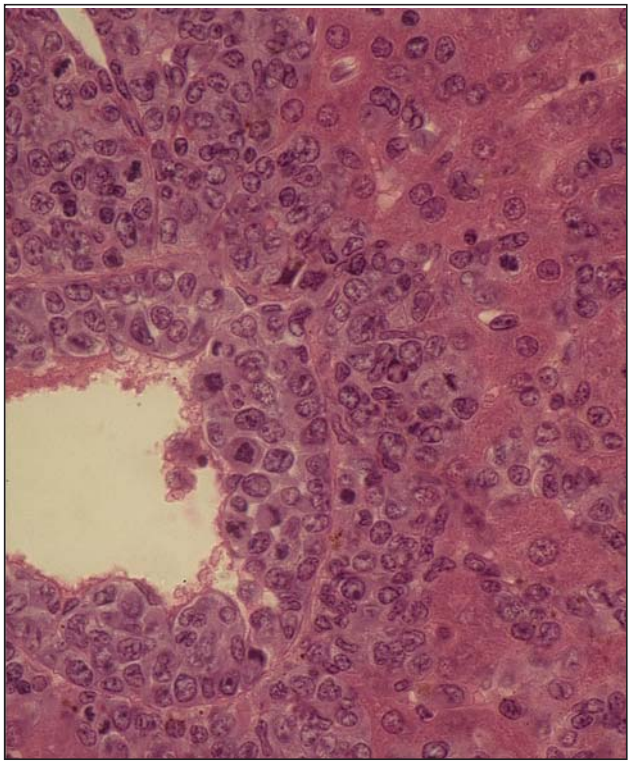

Fig. 7. Perivascular accumulation and angioinvasion of neoplastic myeloid cells in the liver. B210, 17 days. Staining with HE, × 150.

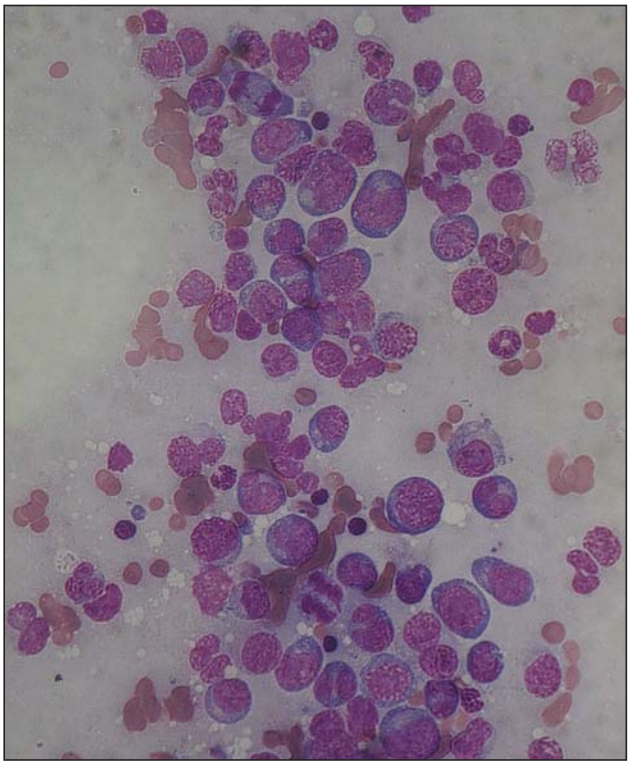

Fig. 8. An imprint of the spleen. Neoplastic cells are large and undifferentiated. The more differentiated cells undoubtedly belong to extramedullary haemopoiesis. B210, 17 days. Staining with Giemsa, × 150.

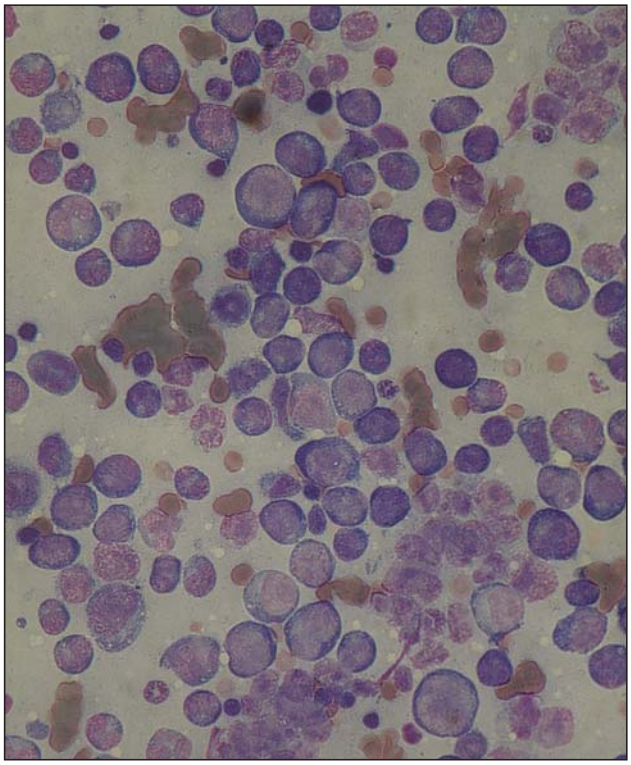

Fig. 9. Imprint of a lymph node. Neoplastic cells are considerably larger than differentiated lymphocytes. No developmental forms of myelopoiesis are present. B210, 17 days. Staining with Giemsa, × 150.

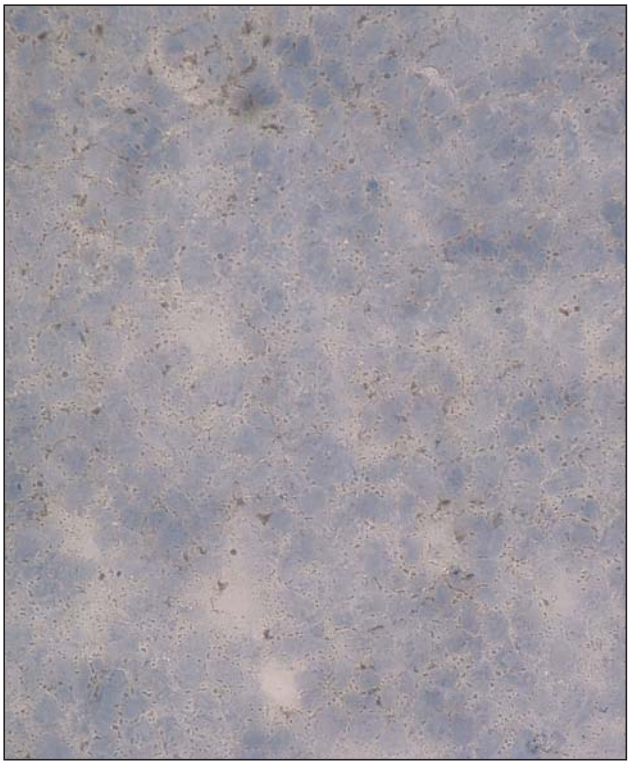

Fig.10. Black cytoplasmic granules in myeloid cells. 12B1, 28 days. Staining with Sudan black B, × 150

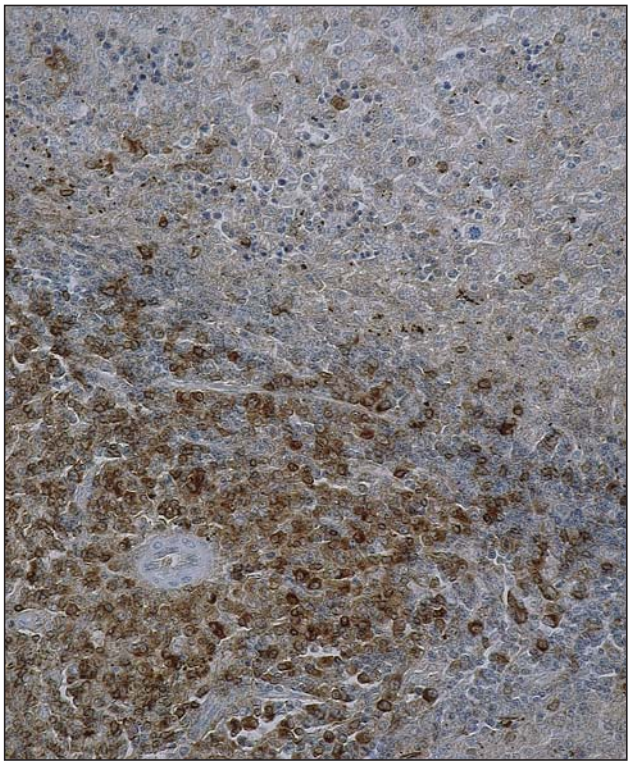

Fig. 11. CD3 in the spleen. Strong positivity in part of the cellular population in PALS and in individual cells in the red pulp. A mild cytoplasmic positivity in myeloid cells. 12B1, 28 days. IPOX, × 80.

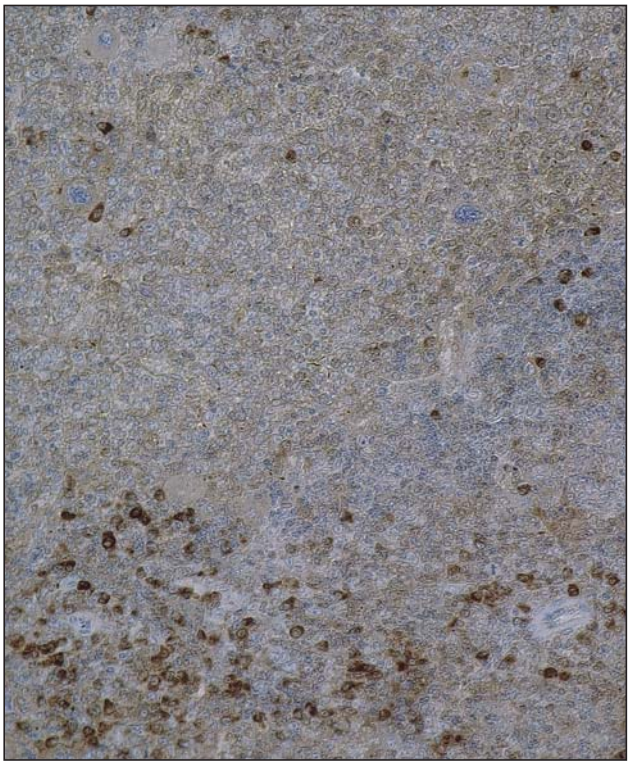

Fig. 12. CD11 in the spleen. Neoplastic meyloid cells show a mild cytoplasmic positivity. Groups of cells with strong cytoplasmic positivity are very probably developmental stages of haemopoiesis. 12B1, 17 days. IPOX, × 80.

Table 3. Summary of histological detection of neoplastic cells

Mouse cells	**1 B210**	**2 B210**	**3 B210**	**4 B210**	**5 B210**	**6 B210**	**7 12B1**	**8 12B1**	**9 12B1**	**10 12B1**	**11 12B1**	**12 12B1**
Brain	++	++	-	+	ND	+	++	++	++	-	ND	ND
Spine	ND	ND	ND	ND	ND	ND	ND	++	+++	ND	ND	ND
Heart	+	+++	ND	-	ND	+	-	+	-	-	-	ND
Lung	+++	++++	ND	+++	+++	++	++	+++	++	++	++	++
Spleen	++++	+++	+++	++++	++++	++++	+++	++++	++++	++++	+++	+++
Lymph n.	+++	++	ND	++	++	+++	-	+++	+++	-	-	-
Thymus	ND	ND	ND	ND	ND	ND	ND	-	ND	-	-	-
Liver	ND	+++	++	+++	+++	++	+++	++	++	++	+	+
Pancreas	++	ND	-	-	-	-	-	-	-	-	-	-
Saliv. gl.	+	ND	ND	-	-	-	-	-	-	-	-	-
Stomach	ND	ND	ND	-	-	-	-	-	-	-	-	ND
Intestine	-	-	ND	-	-	-	-	-	-	-	-	ND
Kidney	++	+++	-	+	+	+	-	++	++	+	+	+
Urin. bl.	-	ND	ND	-	-	-	ND	-	ND	ND	ND	ND
Testicle	ND	ND	ND	-	-*	-	ND	-	-	-	-	-
Uterus	ND	ND	+++	ND	ND	ND	ND	ND	ND	ND	ND	ND
Acces. gl.	ND	ND	ND	-*	-*	-	ND	-	-	ND	ND	ND
Sternum	ND	ND	ND	++++	+++	+++	++++	++++	++++	+++	+++	+++
Vertebra	ND	ND	ND	ND	ND	ND	ND	++++	++++	ND	ND	ND
Soft tisues	ND	ND	ND	ND	ND	ND	ND	+++	+++	ND	ND	ND
Mesentery	ND	+	ND	ND	ND	NF	ND	ND	ND	ND	ND	ND

Legend: - absence of neoplastic cells; + only sporadic neoplastic cells, ++ infiltration of the organ by neoplastic cells without alteration of original structure or presence of some small aggregates of neoplastic cells; +++ severe infiltration of the organ by neoplastic cells with alteration of original structure; ++++ diffuse infiltration of the organ by neoplastic cells with effacement of original structure; NF nodular formations of neoplastic cells of microscopic size in the organ without diffuse infiltration; ND not done; *nodular infiltration of adjacent connective tissue, proper gland free of pathological lesion

Sudan black B staining and enzyme histochemistry

All reactions and staining with Sudan black B were performed both on cryocut and paraffin sections. Only cryocut sections were suitable for these tests. Again, no difference was found between B210 and 12B1 cell lines. Peroxidase was positive in non-neoplastic granulocytes in the form of very small, brown cytoplasmic granules. Myeloid neoplastic cells were negative. Naphthol AS-D chloroacetate esterase was apparent as small blue granules in the cytoplasm of both non- and neoplastic myeloid cells. Non-specific esterase was detected both in non- and neoplastic myeloid cells as a black-dust cytoplasmic positivity. Sudan black B gave the same results as did naphthol AS-D chloroacetate esterase (Fig. 10).

Immunohistochemistry

The results obtained were the same irrespective of whether the animals were inoculated with B210 or 12B1 cells. After treatment with anti-CD3 antibody, numerous cells with a strong cytoplasmic positivity were found disseminated in the red pulp. In PALS, only a portion of the cellular population was positive. Many of these cells were large and of neoplastic appearance. Mild cytoplasmic positivity was observed in the compartments of red pulp infiltrated by myeloid cells (Fig. 11). Only some cells located in liver sinusoids were positive. The findings in lungs were similar.

Using anti-CD79β antibody we detected mild to moderate cytoplasmic positivity in regions of the spleen cellulated by myeloid cells. In PALS formations the results were similar to those seen after treatment with anti-CD3 antibody.

Treatment with CD11b antibody revealed groups of cells or individual cells exhibiting strong cytoplasmic positivity disseminated in the spleens. Some of the cell clusters were in close proximity to structures of PALS appearance. Large areas colonized by cells expressing mild to moderate cytoplasmic positivity were present in the red pulp. These cells were probably both of non-

and neoplastic nature (Fig. 12). Endothelial cells in the liver sinusoids and some cells of neoplastic morphology also displayed reactivity with CD11b antibody. In the lung interstitium only sporadic neoplastic cells were positive.

The findings after treatment with anti-neutrophil antibody strongly resembled those seen after treatment with C11b antibody.

Discussion

Administration of Bcr-Abl-positive cell lines B210 and 12B1 induced in syngeneic mice a disease similar to that described by Pear et al. (1998) and Deng and Daley (2001) as a CML-like syndrome. However, the spleens of our mice displayed a higher degree of variability both in extramedullary haemopoiesis and in the extent of infiltration by neoplastic cells. In adition to spleens, histopathological examination revealed infiltration of many other ogans and tissues with myeloid neoplastic cells. Bone marrow in the sternum was completely or almost completely replaced by neoplastic cells. A similar picture was seen in the lumbar vertebrae. The neoplasia was very aggressive in these locations. The infiltration of bone tissue resulted in osteolysis and spread of neoplastic cells into the surrounding soft tissues, namely the skeletal muscles. The epidural space of the lumbar spine was severely infiltrated by neoplastic cells and it was probably the reason of the paralysis which was clinically observed in three of the 12 mice submitted to pathological examination. It has been mentioned elsewhere (Sobotková et al., 2005) that the hind leg paresis was much more frequent in mice inoculated with 12B1 cells than with B210 cells. In all mice neoplastic cells infiltrated the pulmonary interstitium and in some of them they caused dystelectasis. Apparently, the neoplastic cells used by us have an enhanced tropism for the lung tissue. Moderate or severe infiltration of liver sinusoids was found in nine of 12 animals. In some cases angiotropism and angioinvasion was present. Lesions seen in lymph nodes were apparently of a metastatic nature, because neoplastic cells were first seen in the subcapsular sinuses. Enzyme histochemistry and staining with Sudan black B confirmed the myeloid nature of the neoplastic cells. Immunohistochemical examination for the presence of CD11b and neutrophil antigens revealed a mild positivity in neoplastic cells. Surprisingly, similar results were obtained with anti-CD3 and anti-CD79β antibodies. This could be a consequence of malignant transformation of the primitive multipotent stem cell (Dayley et al., 1991; Li et al., 1999). The CD3 and CD79β reactions also revealed alteration of PALS organization in the spleen.

In general, the findings in mice inoculated with either B210 cells or 12B1 cells were similar. The more aggresive course of the disease in mice inoculated with 12B1 cells, observed in our previous study (Sobotkova et al., 2005), was not reflected in the present histopathological findings.

In the recent past, the disease induced in mice by the administration of cell lines B210 and 12B1 was termed „CML-like syndrome“. On the basis of the present findings we classify the disease induced by B210 and 12B1 cells, in accordance with Valli et al. (2002), as acute myeloid undifferentiated leukaemia. CML in its chronic phase is a myeloproliferative disease characterized by an overproliferation of mature or well-differentiated leukocytes, erythrocytes or platelets, accompanied by blasts in the peripheral blood. In the present experiments we did not observe any maturation of implanted neoplastic cells. On the contrary, we found a progressive infiltration of the spleen by undifferentiated myeloid cells in the majority of mice. Although EMH in the spleen is a physiological process throughout the lifespan in mice, it was rather overextensive in the animals which developed the disease after administration of either of the two Bcr-Abl-positive cell lines. In the experimental mice this process was undoubtedly stimulated by the impairment or absence of haemopoiesis in the bone marrow, which was replaced by neoplastic cells. It is also possible, however, that the extramedullary haemopoieis was stimulated by some cytokines produced by the neoplastic cells.

References

Daley, G. Q., Baltimore, D. (1988) Transformation of an interleukin 3-dependent hematopoietic cell line by the chronic myelogenous leukemia-specific P210 bcr-abl protein. *Proc. Natl. Acad. Sci. USA* **85,** 9312-9316.

Daley, G. Q., Van Etten, R. A., Baltimore, D. (1990) Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. *Science* **247,** 824-830.

Daley, G. Q., Van Etten, R. A., Baltimore, D. (1991) Blast crisis in a murine model of chronic myelogenous leukemia. *Proc. Natl. Acad. Sci.* **88,** 11335-11338.

Deng, M., Daley, G. Q. (2001) Expression of interferon consensus sequence binding protein induces potent immunity against BCR/ABL-induced leukemia. *Blood* **97,** 3491-3497.

Ilaria, R. L., Jr. (2004) Animal models of chronic myeloid leukemia. *Hematol. Oncol. Clin. North Amer.* **18,** 525-543.

Jacobs, R. M., Messick, J. B., Valli, V. E. (2002) Tumors of the hemolymphatic system. In: *Tumors in Domestic Animals,* Fourth Edition, ed. Meuten, D. J., pp. 119-198, Iowa State Press.

Konopka, J. B., Witte, O. N. (1985) Activation of the abl oncogene in murine and human leukemias. *Biochim. Biophys. Acta* **823,** 1-17.

Li, S., Ilaria, R. L., Milion, R. P., Daley, G. Q., Van Etten, R. A. (1999) 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.* **189,** 1399-1412.

Lojda, Z., Papoušek, F. (1970) *Bases of Histochemical Demonstration of Enzymes*. Textbook. Institute for

Postgraduate Education of Medical Personnel, Department for Education of Laboratory Technicians, Brno, CZ, 110 pp. (in Czech)

McLauglin, J., Cjianese, E., Witte, O. N. (1987) In vitro transformation of immature haemopoietic cells by the P210 BCR/ABL oncogene product of the Philadelphia chromosome. *Proc. Natl. Acad. Sci. USA* **84,** 6558-6592.

Pear, W. S., Miller, J. P., Xu, L., Pui, J. C., Soffer, B., Qackenbush, R. C., Pendergast, A. M., Bronson, R., Aster, J. C., Scott, M. L., Baltimore, D. (1998) Efficient and rapid induction of a chronic myelogenous leukemia-like myeloproliferative disease in mice receiving P210 bcr/abl-transduced bone marrow. *Blood* **92,** 3780-3792.

Sobotková, E., Ludvíková, V., Petráčková, M., Dušková, M., Smetana, K., Jelínek, F., Marinov, I., Vonka, V. (2005) Characteristic of two mouse *bcr-abl*-transformed cell lines: I. General properties of the cells. *Folia Biol. (Praha)* **51,** 12-18.

Valli, V. E., Jacobs, R. M., Parodi, A. L., Vernau, W., Moore, P. F. (2002) *Histological Classification of Hematopoietic Tumors of Domestic Animals.* Armed Forces Institute of Pathology, American Registry of Pathology and the World Health Organization Center for Worldwide Reference on Comparative Oncology, p. 190, Washington, DC.

Van Etten, R. A. (2001) Models of chronic myeloid leukemia. *Curr. Oncol. Rep.* **3,** 228-237.