—Review—

Chronic Myelogenous Leukemia: From Molecular Biology to Clinical Aspects and Novel Targeted Therapies

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Abstract

The critical causative event in chronic myelogenous leukemia (CML) is the fusion of the head of the bcr gene with the body of the abl gene, named bcr/abl gene. This chimeric BCR/ABL molecule transforms primary myeloid cells to leukemic cells and induces a CML-like disease in mice. The mouse CML model expressing the BCR/ABL molecule has provided important new insights into the molecular pathophysiology of CML and has directly answered many questions regarding this disease. Furthermore, numerous clinical studies have demonstrated a correlation between leukemic clinical features and the position of the breakpoint in the BCR gene of the chimeric BCR/ABL gene. Understanding of the molecular pathogenesis of CML has led to the development of several novel therapies.

The BCR/ABL molecule is unique oncogeneiety, having ABL tyrosine kinase activity, making it an ideal target for drug development. Subsequent clinical studies now realize the hypothesis that selective inhibition of the abl tyrosine kinase activity using imatinib mesylate might be useful for the treatment of CML.

This article reviews the history of BCR/ABL molecular biology, including the CML model mouse, clinical molecular studies and the recent findings of imatinib mesylate and more potent tyrosine kinase inhibitors developed for the treatment of CML.

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Key words: chronic myelogenous leukemia, imatinib, transgenic mice

Introduction

Chronic myelogenous leukemia (CML) was first recognized as early as 1845[1–3], when several cases of splenomegaly, anemia, and massive granulocytosis were described. Neumann deduced that the disease originated in the bone marrow and proposed the term "myeloid leukemia"[4]. However, it was not until 1960 that the discovery of the Philadelphia (Ph) chromosome led to a better understanding of the pathogenesis of the disease[5]. Thirteen years later, the Ph chromosome was shown to be generated by a specific translocation involving chromosomes 9 and 22[6]. In the 1980s, the BCR-ABL fusion oncogene was described and found to be transcribed and translocated into a functional protein. This novel oncoprotein, P210 BCR/ABL, differed, both in terms of its subcellular localization and its tyrosine kinase activity, from the endogenous c-ABL protein[7,8].

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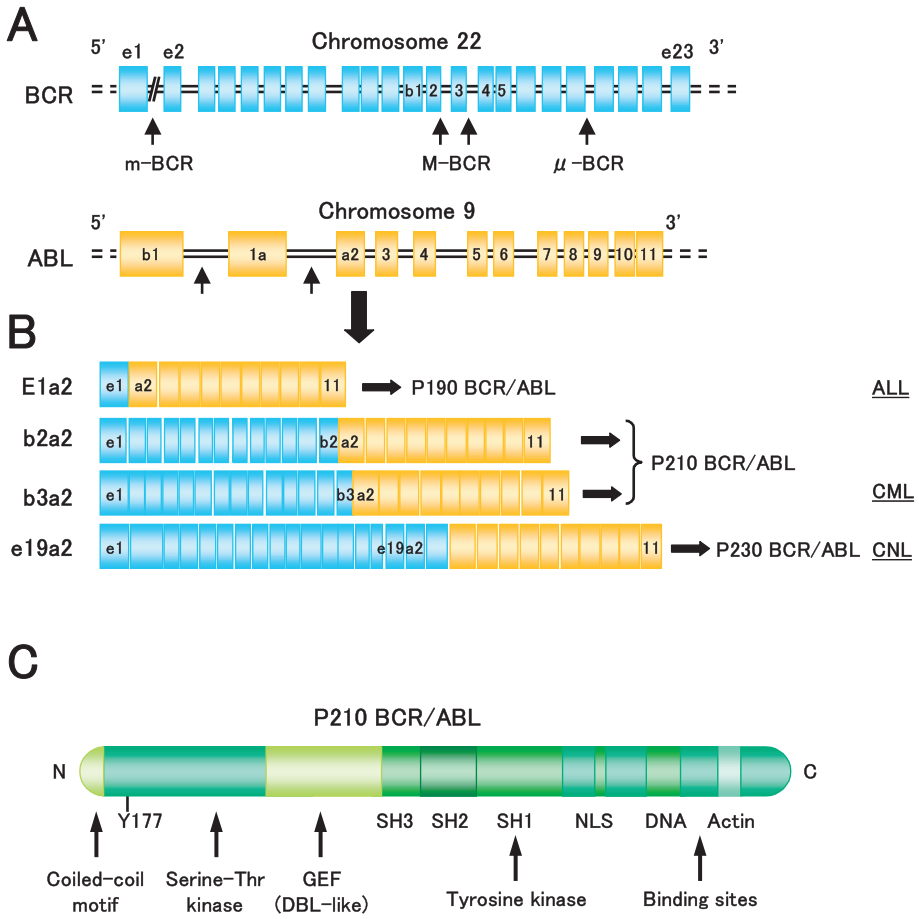

Fig. 1 Three BCR-ABL variants and association of leukemia types. (A) Locations of the breakpoints in the ABL and BCR genes and (B) structure of the chimeric BCR-ABL mRNA transcripts derived from the various breaks. The P185 Bcr/Abl protein is primarily associated with ALL, p210 Bcr/Abl with CML, and P230 Bcr/Abl with CNL. The variants include different amounts of the Bcr sequence, with P230 in cluding the largest amount of Bcr sequence. (C) Functional domains of P210BCR-ABL. Some of the important domains of P210BCR-ABL are illustrated, such as the oligomerization domain (coiled-coil motif), the tyrosine 177 (Grb-2 binding site), the phosphoserine/threonine-rich SH2-binding domain, and the rho-GEF (DBL-like) domain on the BCR portion, and the regulatory src-homology regions SH3 and SH2, the SH1 (tyrosine kinase domain), the nuclear localization signal (NLS), and the DNA- and actin-binding domains in the ABL portion. Abl region of all BCR-ABL variants contains Src-homology (SH3 and SH2) domains. All three variants have a dimerization domain (DD) and serine/threonine kinase domain (P-S/T). P210 and P230 both have DBL and PH domains. In addition, P230 has a calcium/phospholipid binding domain (CalB) and a Gap^{Rac} domain.

Finally, in 1990, a report presented the first definitive evidence of the ability of BCR/ABL to transform primary myeloid cells and induce a CML-like disease in mice[9]. The progress in the understanding of the molecular pathophysiology of CML has led to the development of several novel therapeuetic approaches targeting various steps of the malignant transformation.

1. The BCR/ABL Fusion Protein and Signaling Pathways

Structurally, Bcr/Abl contains many domains **(Fig. 1)**. The Abl sequences encode Src-homology (SH3 and SH2) domains, a tyrosine kinase domain, a DNA-binding domain, an actin-binding domain, nuclear

localization signals, and a nuclear export signal[10]. The Bcr region contains a coiled-coil oligomerization domain, a serine/threonine kinase domain, a pleckstrin homology (PH) domain, a Dbl/cdc24 guanine nucleotide exchange factor homology domain, several serine/threonine and tyrosine phosphorylation sites, and binding sites for the Abl SH2 domain and Grb2[10,11]. The SH2 domain of Bcr/Abl recruits signaling proteins such as p62[dok], c-Cbl, and Rin1[12–14]. Binding and phosphorylation of these molecules may be functionally important as SH2 mutations in Bcr/Abl affect the course of disease in biological models[15]. Expression of the BCR/ABL kinases upregulates cell proliferation[16], decreases apoptosis [17,18], increases cytokine-independent growth[19], decreases adhesion to the bone marrow stroma, and produces cytoskeletal abnormalities[20]. The BCR/ABL fusion protein acts as an oncoprotein by activating several signaling pathways that lead to transformation. Myc, Ras, c-Raf, MAPK/ERK, SAPK/JNK, Stat, NFKB, PI-3 kinase, and c-Jun are included as signal cascade molecules[21–23]. Many signalling proteins have been shown to interact with BCR/ABL through various functional domains/motifs, and/or to become phosphorylated in BCR/ABL-expressing cells. In brief, BCR/ABL activates main signal pathways, such as RAS/MAPK, PI-3 kinase, c-CBL pathways and CRKL pathways, and JAK-STAT, and the Src pathway to play a major role in transformation and proliferation. Inhibition of apoptosis is thought to result from activation of the PI-3 kinase and RAS pathways, with induction through AKT of Myc and BCL-2. The importance of these signalling proteins and pathways in leukemogenesis and their viability as therapeutic targets need to be validated by *in vivo* model systems. Mouse models can be used to determine the involvement of these signalling pathways in pathogenesis and progression of CML.

2. CML Model Mice Expressing the BCR/ABL Gene

The first evidence directly implicating BCR/ABL in the induction of myeloid and lymphoid leukemias came from studies of BCR/ABL transgenic mice[24,25]. A P190 construct induced both acute myeloid leukemia (AML) and B-cell acute lymphoid leukemias (ALL). With a P210 construct, T-cell leukemia also emerged. However, the only model system in which BCR-ABL has induced a CML-like disease was obtained by transplantation into irradiated mice of syngeneic marrow cells that had been retrovirally translocated with BCR/ABL *in vitro*. In the first such experiments, P210 induced a fatal myeloproliferative syndrome in approximately 25% of animals. The CML-like leukemia was a fatal myeloproliferative disease (MPD) arising 4 to 12 weeks following transplantation[9]. Recently, three research groups have modified the original protocol and obtained close to a 100% incidence of transplantable CML, reproducibly and with a short latency that enables study of diseased animals within 4 weeks after transplantation[26–28].

An alternative approach to developing an *in vivo* model of CML has come from the recognition that normal human hematopoietic cells will engraft relatively efficiently in highly immunodeficient mice[29–31]. Engraftment of sublethally irradiated immunodeficient mice has recently been used to analyze the stem cells responsible for causing AML as well as CML[32–34]. A recent report has suggested that, in the NOD/SCID model, engraftment and disease behavior correlate with the donor's CML disease phase. That is, blast-crisis samples engraft early and at high levels and ultimately prove fatal, whereas chronic-phase samples engraft and expand slowly over time, with preferential expansion of leukemic cells after more prolonged periods (> 2 months)[33].

2-1. Conventional BCR/ABL Transgenic Mice

A great deal has been learned from BCR/ABL transgenic mice. Studies of these mice confirm that BCR/ABL can induce leukemia *in vivo*. However, despite more than a decade of work on generating BCR/ABL transgenic mice with conventional techniques, these mice have several drawbacks as a CML model. **Table 1** shows the many kinds of BCR/ABL transgenic mice that have been reported to date.

Results of studies of these CML models suggest

Table 1 History of the transgenic mice expressing BCR/ABL cDNA[92]

Type of BCR/ABLgene	Promoter	Disease phenotype	Author	Publication Year
Bcr/v-abl	MPSV LTR	B,T-ALL	Hariharan, et al.[24]	1989
P190	metallothionein	B-ALL	Heisterkamp N, et al.[25] and Vonken JW, et al.	1990, 1992
P210	bcr	Embryonic lethal	Heisterkamp N, et al.[36]	1991
P210	metallothionein	B,T-ALL	Vonken JW, et al. and Honda, et al.	1995
P190	Bcr (knock-in)	B-ALL	Castellanos A, et al.	1997
P210	m-tec	MPD	Honda, et al.	1998
P210	m-tec	MPD, T-ALL	Honda, et al.[37]	2000
P210	MMTV-LTR-tTA TRE/p210	B-ALL	Huettner H, et al. 2000	2000
P190	metallothionein	B-ALL	Heisterkamp N, et al.	2000
P230	PCMV	MPD	Inokuchi K, et al.[38]	2003
P210	hCD34-tTA TRE/p210	MPD	Huettner CS, et al.[40]	2003
P210	SCLtTA-SV40 TRE/p210	CP of ML	Koschmieder S, et al.[41]	2005

B-ALL: B-cell acute lymphocytic leukemia; T-ALL: T-cell acute lymphocytic leukemia; MPD: myeloproliferative disease; CP of ML: chronic phase of myeloid leukemia.

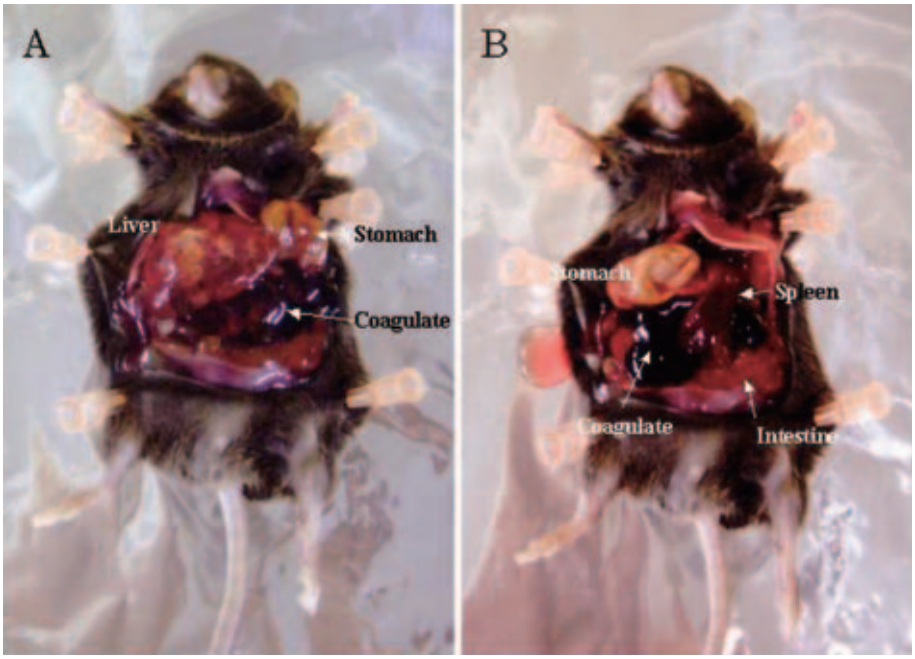

Fig. 2 Macroscopic appearance of founder expressing p230BCR-ABL cDNA at the extramedullary blastic phase of MPD. (A) A markedly enlarged liver and a large hemorrhage in the peritoneal cavity are seen. (B) After removal of the liver, the enlarged spleen is visible.

that P210 BCR/ABL is less potent and has distinct lymphoid leukemogenic activity in transgenic mice when compared with P190. The results also confirmed the leukemogenic activity of P210 BCR/ABL *in vivo*, but none of the P210 BCR/ABL mice reproducibly developed myeloid leukemias or CML-like disease. The reasons a CML-like disease failed to develop are unclear. However, the most plausible explanation may be a lack of sufficient expression of the BCR/ABL transgene in early myeloid progenitor and stem cells, because retroviral expression of BCR/ABL in multipotent progenitor cells does induce MPD in mice[35]. Activated ABL genes have cytotoxic effects, and the presence of the transgene in all tissues raises the possibility of toxicity during embryonic development. The BCR/ABL transgene

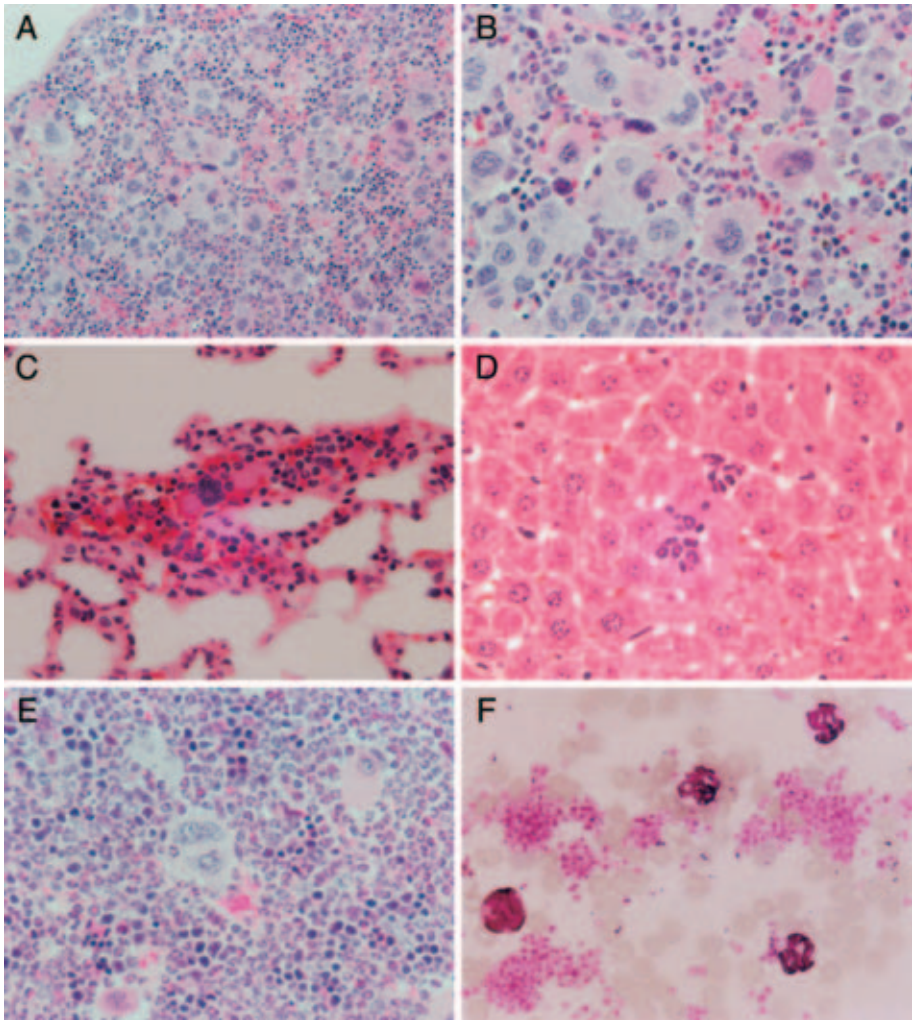

Fig. 3 Histopathologic findings of CML model founder expressed p230BCR/ABL cDNA at chronic phase. (A) and (B) Histopathologic findings of the enlarged spleen. (C) Megakaryocyte were invoved in the lung. (D) The enlarged liver with granurocyte involvement. Marked infiltration of myeloblasts and some megakaryocytes and myeloid cells are seen. (E) Histopathologic findings of the bone marrow. (F) The peripheral blood with granurocytosis and thrombocytosis.

expressed by the BCR promoter caused embryonic lethality[36]. Thus, the P210 BCR/ABL gene was expressed insufficiently in transgenic mice born safely.

After considerable effort, a major breakthrough was finally achieved in 1998. Honda et al. were able to induce CML-like MPD[37]. The P210 BCR/ABL gene is expressed under control of the tec gene as a promoter, which encodes a cytoplasmic kinase preferentially expressed in the hematopoietic system. One reason for the successful creation of MPD model mice may be the particular promoter of the tec gene, which is preferentially expressed in hematopoietic cells. The absence of bcr/abl expression in all tissues except hematopoietic cells may reduce embryonic lethality.

A second successful induction of CML-like MPD was reported in 2003[38]. The reported transgenic mice expressed the P230 BCR/ABL gene under control of the promoter of the long terminal repeat, PCMV, of the murine stem cell virus of the MSCV vector. Two founder mice exhibited MPD mimicking CML **(Fig. 2 and 3)**[37,38]. The level of bcr/abl expression is low in all tissues except hematopoietic cells[38]. This limited expression pattern may permit successful creation of MPD model mice **(Table 2)**.

Table 2 Comparison of hematological and pathological phenotypes of myeloproliferative disease between mouse models expressing p210 and p230BCR/ABL[88]

	p210BCR/ABL (m-tec promoter)	p230BCR/ABL (PCMV promoter)	p210BCR/ABL (hCD34 promoter)
Interval to overt MPD	6-12 months	13-15 months	2-12 months
White blood cell (/mm^3)	$2\text{-}4\times10^4$	$7\text{-}9\times10^3$	$2\text{-}17\times10^3$
Granurocyte rate (%)	>60	20-50	27
Hemoglobin (g/d*l*)	7-9	10-13	12-15
Platelet ($\times10^4$/mm^3)	250-350	150-200	200-600
Cellularity of the bone marrow	Hypercellular	Hypercellular	Hypercellular
Organ of infiltration	Spleen, lymph node	Spleen, liver, lung	Spleen, liver, lymph node
Infiltrating cells	Mature granulocytes	Megakaryocytes, Granulocytes	Megakaryocytes, Granulocytes
Mouse strain	C57BL/6XDBA	C57BL/6N Crj	FVB/N
Reference No.	37	38	40

2-2. Conditional BCR/ABL Transgenic Mice

Recently, attention has turned to the use of conditional transgenic systems as an approach to developing a model of CML, based on the assumption that suppressing expression of a BCR/ABL transgene until after birth would prevent toxicity that might interfere with transgene expression. One type of binary transgene system utilizes the tetracycline-regulated transcriptional activator (tTA).

Double transgenic mice (P210 BCR/ABL-tTA) were generated by breeding female transresponder mice with male mouse mammary tumor virus (MMTV)-tTA transactivator mice under continuous administration of tetracycline[39]. The genotypic distribution of double transgenic mice followed the predicted mendelian frequency. Withdrawal of tetracycline administration to the double transgenic animals allowed expression of P210 BCR/ABL and resulted in the development of lethal B-lymphoid leukemia in 100% of the mice within 1 to 3 months. Rapid complete remission was achieved by suppression of P210 BCR/ABL expression by readministration of tetracycline[39]. In contrast to the MMTV-tTa transactivator line leading to ALL within 2 to 3 weeks after tetracycline induction, the hCD34tTA transgenic line of mice, in which the human CD34 regulatory elements were used to express tTA in bone marrow progenitors, developed MPD characterized by megakaryocytosis (**Table 2**)[40]. The same laboratory reported another new tet-off inducible CML mouse model. In those mice, transgene expression has been successfully induced in the hematopoietic cell compartment by placing tTA expression under the control of the 3' enhancer fragment of the murine stem cell leukemia (SCL) gene[41]. Induction of P210 BCR/ABL resulted in neutrophilia and leukocytosis. Autopsy of the mice demonstrated splenomegaly, myeloid bone marrow hyperplasia, and extramedullary myeloid cell infiltration of multiple organs. Some animals demonstrated a biphasic phenotype consisting of neutrophillia and subsequent B-cell lymphoblastic disease, similar to human lymphoblast crisis. Surprisingly, the interval until the animals become moribund is much shorter than in other reported MPD mouse models. The mice become moribund within 29 to 122 days. This SCLtTA system is a reliable murine model for creating a human CML-like phenotype.

2-3. The Phenotypes of the Disease in Model Mice May Successfully Prove Various Hypotheses

There is a hypothesis that in humans the relative level of BCR/ABL expression may be a determinant in the severity of the phenotype of BCR/ABL-positive leukemia. Patients having P230 BCR/ABL had the disease phenotype of Ph-positive ET or chronic neutrophilic leukemia (CNL)[42–44]. The disease phenotypes of ET and CNL are possibly a result of the expression level of the P230BCR/ABL protein. The difference in the expression level may influence

the disease phenotype in transgenic mice. To investigate this possibility, additional molecular studies will be needed. The transgenic mouse system using PCMV expresses P230 BCR/ABL transcripts at a high level[38]. This evidence of an overt disease phenotype in the P230 BCR/ABL expression model mouse supports the hypothesis.

A second hypothesis is the existence of molecular differences in bcr/abl molecules[45,46]. Molecular differences between p210 BCR/ABL cDNA and p230 BCR/ABL cDNA may be an important disease determinant in both humans and transgenic mice. Both P210 and P230 BCR/ABL contain potential functional motifs encoded by the BCR portion of the fusion gene. The Dbl-like and pleckstrin homology domains are present in the bcr sequence of both P210 and P230 Bcr/Abl[46]. The CalB and GAP^{rac} domains[47,48] in P230 Bcr/Abl may directly influence the ability of this protein to transform various hematopoietic precursors by inhibition of lymphoid development and/or by promotion of myeloid and megakaryocyte development. It is possible that the additional bcr sequences included within P230 BCR/ABL, specifically the GAP^{rac} domain, may partially abrogate the properties of activated p21 Rac in P230 Bcr/Abl-expressing hematopoietic cells. These potential motifs might be involved in the differences in the disease phenotype between P210 BCR/ABL and P230 BCR/ABL transgenic mice.

A third hypothesis is that the type of disease observed in the P210 BCR/ABL model is determined by the type of progenitor cell in which the regulatory cassette is expressed. Several transgenic models of P210 BCR/ABL disease have been generated, but to date there has been no detailed description of the pattern of expression of the transgene in different hematopoietic subsets to correlate with the phenotype.

The disease differences between ALL in the MMTV-tTA system and MPD in hCD34tTA model mice successfully prove that expression of P210 BCR/ABL in early B cells results in B-ALL and that expression in megakaryocytic/erythroid progenitors results in MPD. This mechanism is further supported by studies in which the regulatory elements of the murine SCL gene were used to express tTA in hematopoietic stem cells and myeloid progenitors. Compared with the hCD34tTA system, this SCLtTA system indicates that all transgenic mice develop granulocytosis more typical of chronic-phase CML[41]. These tTA systems successfully show the correlation between the hematopoietic hierarchy, in which BCR/ABL is expressed, and the disease phenotype. Thus, phenotype determination in each patient with CML having the same type of BCR/ABL molecule may depend on the hematopoietic hierarchical populations expressing the BCR/ABL protein.

Recently, another intriguing factor influencing disease phenotype determination was discovered as a result of transplantation of irradiated mice of P210 BCR/ABLretrovirus-transduced marrow cells from knockout mice into irradiated mice[49]. This factor consists of three Src kinases, Lyn, Hck and Fgr. The transplantation of P210 Bcr/abl expressing hematological stem cells from triple knockout mouse lacking all three Src kinases induced a CML phenotype but not ALL in the recipient mice. These results indicate Src kinases are important factors in determining whether the disease phenotype is CML or ALL.

3. The BCR/ABL Genes and Disease Phenotype

3-1. The BCR/ABL Genes and Leukemic Phenotype (Table 3 and Fig. 1)

Bcr/Abl oncogenes differ in the amount of Bcr included in the fusion protein. This difference in structure influences the biological and clinical phenotypes associated with the Bcr/Abl variants[50]. Splicing at the m, M, or μ breakpoints in Bcr produces three distinct proteins. These three BCR/ABL variants are named P190 (e1a2 junction), P210 (b2a2 or b3a2 junction), and P230 (e19a2 junction) **(Fig. 1)**[50]. P190 encodes the dimerization and SH2-binding domains of Bcr[51,52]. P210 has the Bcr PH and Dbl domains in addition to the domains present in P190[53]. Finally, P230 has the largest Bcr sequence, including the calcium/phospholipid binding domain and the first third of the domain associated with GTPase activity for p21 (GAP-Rac)[54].

Although the three Bcr/Abl kinases (P190, P210,

Table 3 The different types of leukemia and the corresponding BCR/ABL breakpoints

BCR breakpoint	Junction BCR/ABL mRNA	BCR/ABL molecule	Disease phenotype*
Minor (m)	e1/a2	P190BCR/ABL	ALL
Major (M)	b2/a2, b3/a2	P210BCR/ABL	CML
Micro (μ)	e3/a2	P230BCR/ABL	CNL, CML with thrombocytosis

ALL: acute lymphoblasti leukemia; CML: chronic myelogenous leukemia; CNL: chronic neutrophilic leukemia. *: major disease phenotype is described.

Table 4 Comparison of bcr breakpoint data, mRNA data and platelet count[89]

Number of patients	Mean platelet count ($\times 10^9$/L)				Reference
	Hind III restriction site of M-bcr		Subtype of Bcr/Abl transcript		
	5' Breakpoint	3' Breakpoint	b2/a2	b3/a2	
57			374	842	64
62	302	448			86
22	274	1395			65
247	387	490			87
64			357	501	87
119	530	537	395	534	63
88			306	616	66
70			470	545	67

and P230) have similar in structures, they exhibit distinct properties. When primary mouse bone marrow cells are infected with the different Bcr/Abl variants and cultured in the presence of cytokines and stroma, P190 cultures differentiate into a lymphoid lineage, whereas P210 and P230 become myeloid[53]. In vitro, P190 has a greater ability to stimulate expansion of lymphoid cells than does P210[55]. P230 Bcr/Abl cultures require cytokines for optimal growth, whereas P190 and pP210 Bcr/Abl cultures are cytokine-independent for growth and survival[53]. P190 Bcr-Abl has a greater tumorigenic potential than does P210 or P230 Bcr/Abl[53]. In soft agar assays, P190 is 100-fold more effective than P210 at eliciting transformation[56]. There are several possibilities to explain the different biological effects of the three BCR/ABL kinases. Intrinsic tyrosine activity is lower in P230 than in P190 or P210[28]. P210 and is lower in P210 than in P190[56]. The SH2 domain is required for efficient induction of CML-like disease in mice by P210 but not by P190, further suggesting a difference in signaling between the two variants[18].

Differences in the content of the Bcr region itself might lead to distinct clinical disease[57]. Additional Bcr sequences, such as Dbl/PH in P210 and Gap^{Rac} in P230 may allow for increased differentiation along the myeloid lineage[50,57]. The Dbl/PH domains present in P210 but not P190 may also contribute to the stabilization of actin fibers[59]. Dbl activates Rho, and activated Rho promotes the formation of actin stress fibers[60]. Alternatively, it is possible that Bcr breakpoints that yield P 190 might occur preferentially in immature B cells, and breakpoints that produce P210, in hematopoietic stem cells[57].

3-2. Breakpoints within M-bcr and the Prognosis Correlation in CML

Due to the proximity between the *Hind* III restriction site of the M-bcr region and the BCR exon 14 **(Fig. 1)**, the comparison of the data of Southern blot with those of RT-PCR indicated that a significant proportion of the patients with the 5' breakpoint in the M-bcr had a b3a2 variant of the transcript, which is a consistent feature of the 3' breakpoint group in p210BCR/ABL **(Fig. 1)**. Regarding the prognosis, the chronic phase lasted fourfold longer in patients with breakpoints located upstream to the central *Hind* III restriction site (5' breakpoint) than in patients with 3' breakpoints[61]. This finding was not finally confirmed in subsequent

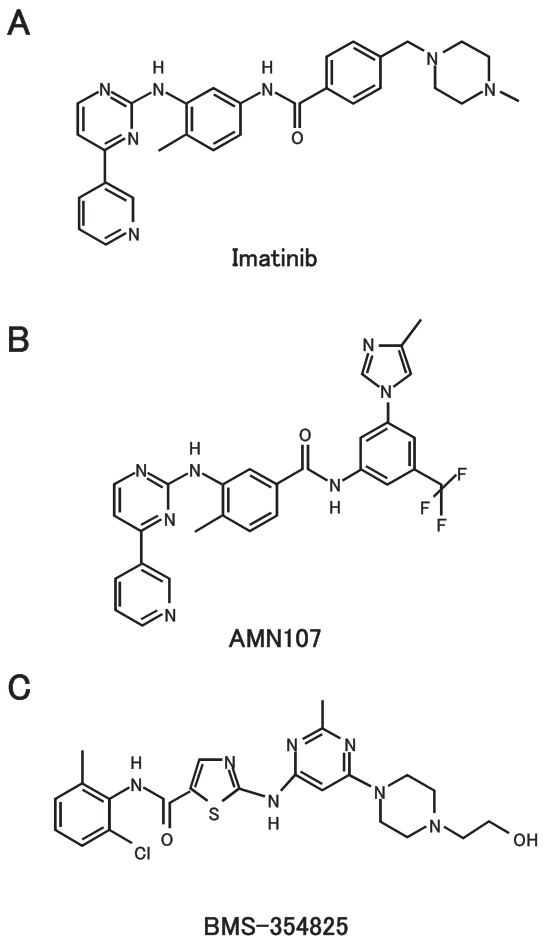

Fig. 4 Novel BCR/ABL tyrosine kinase inhibitors. the ABL tyrosine kinase inhibitors (A) imatinib, (B) AMN107 and (C) BMS-354825.

series of patients, including those reported in very large studies[62,63].

3-3. M-bcr Breakpoints and Higher Platelet Number in CML (Table 4)

A matter of discussion is the correlation between the breakpoint location within the M-bcr and the platelet count. Two intriguing studies have reported statistically significant increases in platelet counts in patients with the 3' breakpoint or the b3a2 transcript[64–66]. The majority of studies in which the platelet count was stratified according to the breakpoint position in the M-bcr region or to the type of transcript showed higher platelet counts in patients with the b3a2 transcript or the 3' breakpoint[67,68]. The findings of two reports were, at least in part, confirmed by data from patients with CML and low WBC counts at presentation. In a series of 23 patients with WBC<100 × 10^9/L[63,66] mean platelet count in patients with the b3a2 type (653 × 10^9/L) were significantly higher than those in patients with the b2a2 type of transcript (381 × 10^9/L). In addition, two different studies investigated the type of transcript in a total of 18 patients with thrombocytemic onset of CML and found the b3a2 transcript in 13 cases[69,70]. The negative correlation between the WBC count with platelet count and the length of BCR sequence included in the BCR/ABL fusion gene is in accordance with the high frequency of thrombocytosis found in the patients with CML[71]. The reviewers, Pane et al., have concluded that the length of BCR sequences included in the fusion protein is positively correlated with the platelet count[72] (**Table 4**).

4. Imatinib Mesylate; STI571

4-1. Targeting ABL with Imatinib Mesylate (Fig. 4)

An improved understanding of the molecular mechanism involved in the development of CML has led to the development of targeted therapies. Druker and colleagues developed the experimental drug CGP57148B, which is known as imatinib mesylate (imatinib, STI571, Gleevec/Glivec). Imatinib is an ABL tyrosine kinase inhibitor of the 2-phenylamino pyrimidine class that was created using the structure of the ATP binding site of the ABL protein kinase[73,74]. Imatinib binds to and stabilizes the inactive form of BCR/ABL rather than occupying the whole ATP-binding pocket as previously believed[75]. Imatinib voids the effects of the BCR/ABL oncoprotein through inhibition of BCR/ABL autophosphorylation and substrate phosphorylation, inhibition of proliferation, and induction of apoptosis[73]. Targeted therapy with imatinib has led to a revolution in the treatment of CML. A phase I clinical trial of this drug was conducted in 1998, and 98% of patients with interferon-resistant CML in the chronic phase achieved a complete hematologic response and 31% of these patients achieved a complete or major cytogenetic response. The following phase II study confirmed these impressive results. Ninety-five percent of patients in the chronic

phase can achieve a complete hematologic remission and 60% can achieve a major cytogenetic response with imatinib. Generally, imatinib has been well tolerated in patients with CML, and in patients with other BCR/ABL-positive leukemias[76]. Imatinib is well absorbed after oral administration with a mean absolute bioavailability for the capsule formulation of 98%. Following oral administration, the elimination half-lives of imatinib and its major active metabolite, the *N*-desmethyl derivative, are approximately 18 hours and 40 hours, respectively[77].

4-2. Mechanisms of Resistance to Imatinib

Although high rates of complete remission have been observed with imatinib in patients with chronic-phase CML, a short duration of respose with eventual emergence of Imatinib resistence has also been observed in some patients with CML. Resistance to imatinib can be defined as the lack of complete hematological response in patients with chronic-phase disease or as a lack of return to the chronic phase for patients in the acute phase, in blast crisis CML.

4-2-1. Point mutations within ABL kinase

Potentially the most frequent clinically relevant mechanisms that change imatinib sensitivity in BCR/ABL transformed cells are mutations within the ABL kinase, affecting several of its properties. Point mutations can directly influence the proper binding of imatinib to ATP-binding kinase domain of BCR/ABL. Mutations can lead to conformational changes of the protein, indirectly affecting binding of either imatinib or ATP in an indirect way. Imatinib-resistant mutations are likely to be induced by imatinib itself, due to selection of BCR/ABL-expressing clones that harbor the point mutation. To date, more than 30 different point mutations encoding for distinct single amino acid substitutions in the BCR-/ABL kinase domain have been identified in 50 to 90% of patients with relapsed CML[78,79]. Mutations of the kinase domain can lead to decreased sensitivity of imatinib by 3-fold to more than 100-fold. The decrease in imatinib sensitivity is heterogeneous and varies between the distinct mutations.

4-2-2. Imatinib resistance by Bcr/Abl gene amplification

Resistance to imatinib can also be caused by overexpression of the BCR/ABL protein due to amplification of the Bcr/Abl gene. This mechanism was initially described in the Bcr/Abl-positive cell line with a 4.6-fold increase in mRNA levels[80]. This mechanism of resistance is observed in a small percentage of the patients with Imatinib resistance[78] and can be detected by interphase fluorescence in situ hybridization using fluorescently labeled probes for the Bcr and Abl genes[78,81].

4-2-3. Pharmacological mechanisms

Imatinib needs to pass through the plasma membrane to reach its target protein, like most other small-molecule drugs. In many cases of drug resistance, transmembrane proteins involved in ion transport across the plasma membrane (or pumps) have been implicated in mediating drug resistance. It appears that this mechanism plays a minor role in Imatinib-related resistance.

4-2-4. BCR / ABL-independent cytogenetic aberrations in imatinib resistance

BCR/ABL has been associated with genomic instability, which may have particular relevance during disease progression from the chronic phase to accelerated and blast-phase CML. Many studies have described additional mutations in Ph+ cells; however, there does not appear to be a particular mutation in addition to the t (9 ; 22) (q34 ; q11) translocation that drives transformation in any specific way. Accumulation of additional mutations might be sufficient for transformation by themselves, independent of BCR/ABL.

5. Novel ABL Kinase Inhibitors Targeting Imatinib Resistant BCR/ABL

The expanded knowledge of the different mechanisms of imatinib resistance has helped the development of strategies to solve these problems. One goal is to identify new compounds that bind to and inhibit ABL kinase but are less affected by point mutations through their static conformation. In particular, crystal structure analysis of the ABL-imatinib complex has proven helpful in identifying potential critical residues that hinder interaction of

imatinib with mutated Abl[82]. The strength of inhibition might also be increased by identifying compounds that can bind to the active and inactive ABL kinase conformation. Another strategy has been to target the substrate-binding pocket in ABL. I will summarize recent findings about three novel drugs as well as a variety of dual-specific Src/ABL inhibitors that have been developed using different strategies.

5-1. AMN107, a Cousin of Imatinib (Fig. 4)

Several recent preclinical studies have shown promising results for the second-generation ABL inhibitor AMN 107, developed by Novartis Pharmaceuticals (Basel, Switzerland). Imatinib was developed through rational drug design, and based on its success, the structurally related aniliopyrimidine derivative AMN107 was created **(Fig. 4)**[83]. Due to this structural similarity between the two compounds, AMN107 also requires the ABL protein to be in the inactive conformation for optimal binding. The pharmacological profile of AMN107 towards the wild-type BCR/ABL protein and several imatinib-resistant BCR/ABL proteins with point mutations has already been established. Using numerous BCR / ABL transformed hematopoietic cell lines, AMN107 was found to be 10- to 25-fold more potent than imatinib in reducing both autophosphorylation and proliferation[83]. Many imatinib-resistant Bcr/Abl mutations might be effectively targeted by AMN107; however, clones carrying the Y253H, E255V, and T315I mutations are all markedly resistant, even at very high *in vitro* doses.

5-2. BMS-354825, a Novel ABL and SRC Family Tyrosine Kinase Inhibitor (Fig. 4)

The pyridol [2,3-d] pyrimidine BMS-354825 is a novel ABL-targeted small-molecule inhibitor developed by Bristol-Myers Squibb (Princeton, NJ, USA) that also shows activity towards Src kinases. Shah et al. have recently demonstrated that BMS-354825 has up to 100-fold increased activity against the ABL kinase than does imatinib and retains activity against 14 of 15 imatinib-resistant Bcr/Abl mutants *in vitro*[84]. The T315I substitution is the only known BMS-354825-resistant mutant[84] and, as discussed above, has been shown to be resistant to AMN107 in preclinical studies.

5-3. ON012380, a Substrate Binding Site ABL Inhibitor

Unlike imatinib, the ABL inhibitor ON012380 was specifically designed by Onconova Therapeutics (Princeton, NJ, USA) to block the substrate binding site rather than the ATP binding site[85]. This strategy has the advantage in that the previously described imatinib-resistant mutants are unlikely to be resistant to this inhibitor, due to the different binding sites. In vitro studies confirmed this assumption and ON012380 was able to inhibit both wild-type and all imatinib-resistant kinase domain mutations, even the problematic T315I mutation with an IC_{50} of less than 10 nM. Interestingly, ON 012380 also has activity against the PDGFR kinases and the Src family member Lyn with IC_{50} values for inhibition of proliferation of approximately 80 nM. However, inhibition of the c-kit kinase is weaker.

6. Conclusion

The molecular mechanism accounting for the relationship among the different types of the Bcr/Abl gene and the leukemic phenotypes are a fascinating issue in molecular hematology but remain largely obscure. However, some data suggest that, besides the intrinsic tyrosine kinase activity of BCR/ABL proteins, even their levels within the Ph-positive precursors may play an important role in determining the severity of the hematological conditions. Much progress has been made in the understanding of the molecular pathophysiology underlying CML and has led to the development of targeted and effective therapies. With the introduction of imatinib, major advances in the treatment of CML have been achieved. However, as with many other anti-cancer drugs, clinical resistance to imatinib monotherapy has emerged. The need for alternative or additional treatments has lead to a second generation of targeted therapies, resulting mainly in the development of novel small-molecule inhibitors, such as AMN107,

Table 5 Novel targeted therapies for CML

1. transcript of Bcr/Abl: siRNA, antisense oligodeoxynucleotides, ribozymes
2. tyrosine kinase: imatinib, AMN107, BMS-354825, N012380
3. Ras pathway:
   farnesyl transferase inhibitors: R115777, SCH66336, BMS-214662
   MAP kinase inhibitors: U0126
4. Phosphatidyl inositol-3'-kinase (PI3K) pathway
   PI3K inhibitors: LY294002, wortmannin
   phosphoinositide-dependent kinase-1 (PDK-1) inhibitor: OSU-03012
   The mammalian target of rapamycin (mTOR) inhibitor: rapamycin, RAD001, CCI-779, AP23573
5. Other targets
   histone deacetylase inhibitors: suberoylanilide hydroxamic acid (SAHA), proteasome inhibitors (bortezomib)
   heat shock protein inhibitors: benzoquinone ansamycins (geldanamycin, 17-allylamino-17-demethoxygeldanamycin [17-AAG])

BMS-354825 and ON012380. The development of inhibitors of other signal-transduction proteins, such as farnesyl transferase inhibitors, may allow additional therapeutic alternatives (**Table 5**). These new approaches may provide strategies to treat CML and other leukemias.

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