Chronic myeloid leukaemia as a model of disease evolution in human cancer

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Abstract | Chronic myeloid leukaemia (CML) can be considered as a paradigm for neoplasias that evolve through a multi-step process. CML is also one of the best examples of a disease that can be targeted by molecular therapy; however, the success of new 'designer drugs' is largely restricted to the chronic phase of the disease. If not cured at this stage, CML invariably progresses and transforms into an acute-type leukaemia undergoing a 'blast crisis'. The causes of this transformation are still poorly understood. What mechanisms underlie this progression, and are they shared by other common cancers?

Chronic phase
The initial stage of CML, in which most patients are diagnosed. It usually has an insidious onset, and the main clinical findings include enlarged spleen, fatigue and weight loss. The peripheral blood shows leukocytosis (approximately 150×10^9/L white blood cells (WBCs)), predominantly owing to neutrophils in different stages of maturation, as well as basophilia and eosinophilia. Blasts usually represent <2% of the WBCs. The platelet count is normal or increased.

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For various reasons, chronic myeloid leukaemia (CML) is probably one of the most comprehensively studied human malignancies. CML was the first human cancer to be associated with a consistent chromosomal abnormality — the Philadelphia (Ph) chromosome — a balanced, reciprocal translocation involving the long arms of chromosomes 9 and 22 (REFS 1,2). CML is characterized by distinct clinical phases: most patients present in chronic phase (CP), a phase in which mature granulocytes are still produced, but patients have an increased number of myeloid progenitor cells in the peripheral blood. As the disease progresses, patients enter an accelerated phase (AP) followed by blast crisis (BC), in which haematopoietic differentiation has become arrested and immature blasts accumulate in the bone marrow (BM) and spill into the circulation. The CP is relatively long-lasting, so researchers have the opportunity to study malignant cells with an 'indolent' behaviour and to identify the changes associated with transformation to the 'aggressive' phenotype of blast crisis. Furthermore, CML is unusual in that a single genetic lesion, or 'hit', occurring in a haematopoietic stem cell generates a fusion oncogene, *BCR-ABL*, which encodes a protein tyrosine kinase that is necessary and sufficient for cell transformation[3–5]. Access to the neoplastic clone is relatively straightforward without the need for invasive surgery, which simplifies the collection of samples for studying the disease. Finally, CML was the first haematological malignancy for which a programme of rational drug design yielded an effective targeted molecular therapy (imatinib mesylate, Gleevec or Glivec)[6,7]. In this Review we consider how the current state of knowledge regarding the biology of advanced phase CML compares with other human cancers. In particular, we discuss those features that, if not universal, are still relevant for most human cancers, including differentiation arrest, genomic instability (which includes failures of genome surveillance, deficiencies of DNA repair and mutator phenotype), telomere shortening and the loss of tumour-suppressor functions (FIG. 1; TABLE 1). The role of gene-expression profiling as a means of identifying candidate genes involved in disease progression is also highlighted.

BCR-ABL and the leukaemic clone

BCR-ABL is generated by a t(9;22)(q34;q11) translocation that juxtaposes the 3′ sequence from the *ABL1* proto-oncogene on chromosome 9 (REF. 8) with the 5′ sequence from the *BCR* gene on chromosome 22 (REFS 9,10). Proof that *BCR-ABL* was the causative agent of CML was provided by a murine model in which mice transplanted with bone marrow retrovirally transduced with *BCR-ABL* developed a myeloproliferative syndrome that recapitulated features of CML[5].

Molecular signalling in CML is highly complex, and the signal transduction pathways affected by BCR-ABL have been extensively reviewed elsewhere (see REF. 11). The cytoplasmic location of the BCR-ABL oncoprotein[12] allows access to many cellular substrates that are unavailable to the predominantly nuclear ABL protein[13], determining the activation of proliferation and survival pathways. However, the true relevance of BCR-ABL itself in causing disease progression is uncertain[14]. In BCR-ABL-expressing cell lines, secondary mutations that cause the constitutive activation of the STAT5 (signal transducer and activator of transcription 5) pathway[15–18] can maintain the transformed phenotype when *BCR-ABL* is silenced[19]. However, with rare exceptions, patients with CML retain the expression of *BCR-ABL* in the leukaemic clone throughout the course of the disease,

At a glance

- Chronic myeloid leukaemia (CML) can be considered, for some aspects, a model for malignant tumours that evolve through a multi-step pathogenetic process. In its natural history, CML is usually diagnosed in chronic phase (CP) and then progresses through an accelerated phase to a nearly invariably fatal blast crisis (BC).
- The mechanisms of transformation to BC are varied and not entirely understood. So far the best characterized include differentiation arrest, genomic instability, telomere shortening and loss of tumour-suppressor functions.
- The differentiation arrest of the transformed BC clone is caused by the suppression of translation of the transcription factor CEBPα induced by the BCR-ABL oncoprotein in CML cells, through increased stability of the heterogenous nuclear ribonucleoprotein E2 translational regulator.
- The increased genomic instability in CML cells is the result of their reduced capacity to survey the genome for DNA damage and to correctly repair DNA lesions, which leads to the accumulation of deleterious mutations in genes that are essential for maintaining normal cell physiology and maturation.
- The impairment of several tumour-suppressor genes has been variably associated with BC of CML, including *TP53*, retinoblastoma 1, *CDKN2A* and others. Protein phosphatase 2a (PP2A) has an important role, as it is inhibited by BCR-ABL in BC cells by the post-transcriptional upregulation of SET, a phosphoprotein that is frequently overexpressed in other leukaemias and solid tumours.
- Recent expression profiling of cells from different stages of CML has uncovered several genes that are differentially expressed in BC and in extremely aggressive forms of the disease. These are likely to be associated with the kinetics of blastic transformation.
- It is envisaged that the identification of the most pathologically relevant genetic lesions for the development of BC will allow the early diagnosis of impending disease progression and the design of new treatment strategies to halt this process. Such an approach can become the paradigm for therapy decision making in other types of cancer.

Accelerated phase
An intermediate stage of CML evolution, when the disease starts to become refractory to therapy. It is characterized by an increase in spleen size and in total WBCs, blasts comprising 10–19% of the WBCs, >20% circulating basophils, persistent thrombocytopenia and/or the appearance of new clonal cytogenetic abnormalities.

Blast crisis
The final stage of CML, which may or may not be preceded by an 'accelerated phase'. Patients experience worsened performance status, and symptoms related to thrombocytopenia, anaemia and increased spleen enlargement. The WHO (World Health Organization) criteria for the diagnosis of blast crisis include: blasts in excess of 20% in the peripheral blood or bone marrow; and/or extramedullary blast proliferation; and/or large foci or clusters of blasts in bone marrow histological sections.

suggesting that BCR-ABL must be important for the continued maintenance of the neoplastic phenotype, even in the advanced phase, and that a selection pressure favours the continued activity of the oncoprotein. In fact, increased BCR-ABL expression is likely to contribute to the phenotype of advanced phase disease, as studies using cell line models of CP and BC CML indicate that the oncoprotein exerts dose-dependent effects on growth factor dependence[20–22], clonogenicity[20,22], migration[22] and the rate at which cells develop resistance to imatinib[23]. Moreover, the expression levels of BCR-ABL increase with disease progression[24–27], a phenomenon that has recently been shown to occur at the mRNA and protein levels in $CD34^+$ progenitors[22]. Significantly, this population includes the immature, self-renewing cells that predominate in CML BC. Differentiation arrest and the inappropriate reactivation of self-renewal capacity are two aspects of disease evolution in CML.

Differentiation arrest

Abnormal or deficient differentiation is common in haematological malignancies and solid tumours with poor prognosis[28]. CML is a good example of a cancer in which the transition from mature, terminally differentiated cells to immature, undifferentiated cells can be observed in the malignant clone. This differentiation arrest implies pathological interference with differentiation 'programmes' involving the targeted activation of tissue-specific genes by transcription factors. Such interference may be instigated by oncogene products, as has been demonstrated for the suppression of the transcription factor CEBPα by BCR-ABL[29] (FIG. 2).

CEBPα is a member of the basic leucine zipper family of transcription factors, and activates the transcription of the granulocyte colony-stimulating factor receptor (*GCSFR*) and *ID1* genes in myeloid cells[30,31]. Although CEBPα is expressed in normal BM and CML CP samples, it is undetectable in CML BC cells[29]. BCR-ABL suppresses the translation of CEBPα by increasing the stability of the translational regulator, heterogeneous nuclear ribonucleoprotein E2 (HNRNPE2)[29]. Binding of HNRNPE2 to CEBPα mRNA inhibits the subsequent translation of the transcription factor. Expression of HNRNPE2 is inversely correlated with that of CEBPα in BCR-ABL-expressing cells and is readily detectable in CML BC samples but is low or undetectable in normal and CML CP BM cells[29]. The abrogation of *CEBPα* translation owing to the induction of HNRNPE2 by BCR-ABL may prove to be a key event responsible for the differentiation arrest that occurs in CML[29], highlighting the importance of epigenetic mechanisms, such as post-transcriptional repression, in oncogenesis.

Although inactivating mutations of *CEBPα* would cause a similar undifferentiated phenotype as in acute myeloid leukaemia (AML)[32], a recent study[33] has found that such mutations are not a feature of CML. Consistent with a role of CEBPα in promoting differentiation, recent reports indicate that its ectopic expression restores differentiation in BCR-ABL-transformed cell lines or cells from patients with BC CML[34,35]. In addition, although mice transplanted with BCR-ABL-expressing $Cebp\alpha^{+/+}$ fetal liver cells developed a CML-like myeloid disease showing the requirement of CEBPα for granulopoiesis, mice transplanted with BCR-ABL-expressing $Cebp\alpha^{-/-}$ cells developed erythroleukaemia[31,36], indicating that a residual level of CEBPα seems to be required for the malignant haematopoiesis associated with BC of the myeloid phenotype[31].

Another mechanism of differentiation block in CML myeloid BC is the effect of mutations or gene translocations that result in the formation of dominant-negative transcription factors, such as *AML1–EVI1* (REF. 37) or *NUP98–HOXA9* (REF. 38) fusion genes, which have been described in a few isolated cases of myeloid BC[39–42]. Although the precise mechanisms responsible for the differentiation arrest in these cases are unknown, the truncation of both transcription factors seems to act cooperatively with BCR-ABL to interfere with the signalling required for the correct activation of differentiation programmes.

Cell of origin of BC. Another aspect of the maturation arrest in BC is the question of whether the transformed subclone originates from a cell that is at a distinct differentiation stage from that which gives rise to CP. Thus, disease progression in CML may originate in more committed precursors than had been previously supposed, as myeloid BC has been reported to involve

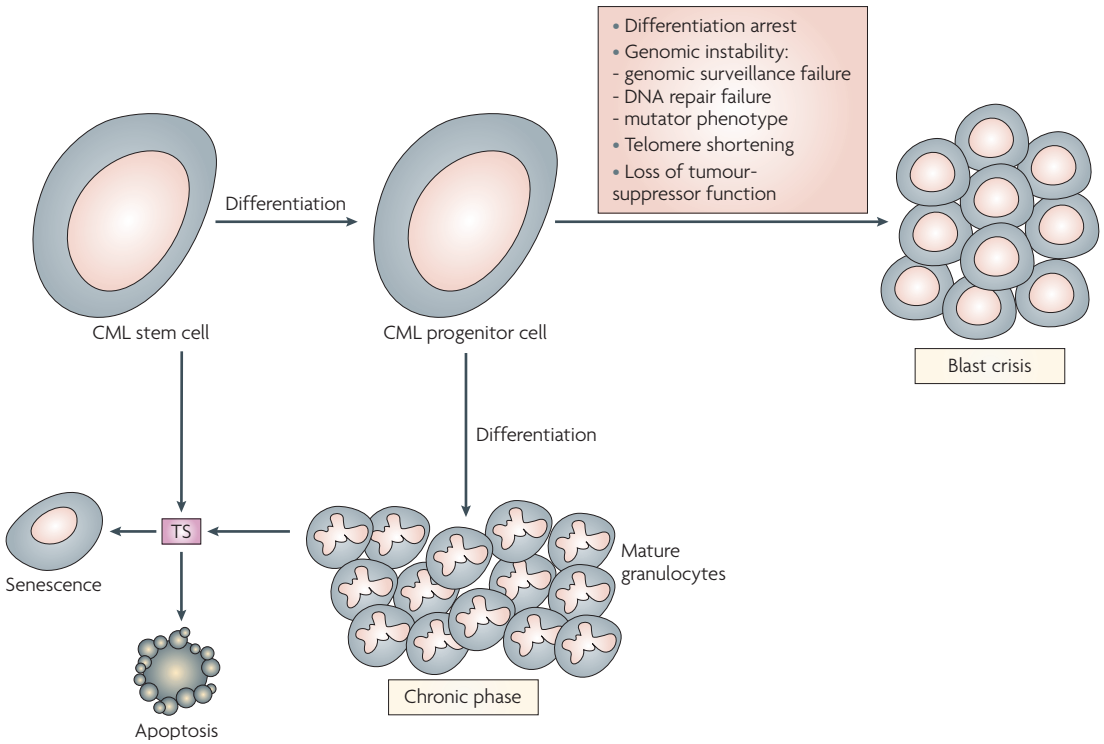

Figure 1 | **Disease progression in chronic myeloid leukaemia.** In chronic phase (CP), the bulk of leukaemic stem cells remain capable of undergoing differentiation, leading to the excessive production of mature granulocytes. In advanced phase disease, differentiation has become arrested, probably at the stage of the leukaemia progenitor cell, and the 'aggressive' disease phenotype is caused by the proliferation (self-renewal) of immature blasts. Deleterious genetic events (inset) are believed to accumulate within stem and progenitor cells of the leukaemic clone until there are sufficient secondary mutations to drive the transition from chronic to advanced phase disease. These include: an increase in genomic instability through interference with genomic surveillance and DNA-repair proteins and a progressive telomere shortening. In CP cells essential tumour-suppressor (TS) proteins remain functional and allow cells to undergo replicative senescence or apoptosis. However, in advanced phase blasts there is evidence that TS function has been lost.

Mutator phenotype
A mutator phenotype arises owing to mutations in genes that are crucial for maintaining genomic stability.

Telomere
The termini of eukaryotic chromosomes that function to prevent the loss of genetic material. Human telomeres consist of tandem repeats of the DNA sequence TTAGGG. Telomerase is a ribonucleoprotein, required for the maintenance of telomeres, consisting of an RNA template and a catalytic subunit (TERT in humans). Whereas human stem cells express telomerase and retain intact telomeres, most somatic cells do not, and consequently have telomeres that become shorter with successive cell divisions.

STAT5
A transcription factor that is activated by multiple haematopoietic cytokines. In cell lines, BCR-ABL was shown to catalyse the phosphorylation of tyrosine residues on STAT5, leading to its constitutive activation. STAT5 promotes cell survival and proliferation by transactivating anti-apoptotic genes (*BCL-X*$_L$) and genes associated with cell-cycle progression (cyclin D1).

CD34
A cell-surface protein expressed by haematopoietic stem cells, haematopoietic progenitor cells and endothelial cells, which is used as a marker to isolate human haematopoietic progenitor cells.

the granulocyte-macrophage progenitor (GMP) 'pool' rather than the haematopoietic stem cell pool[43]. The self-renewal of GMPs requires the activation of the β-catenin pathway[43]. In mouse haematopoietic stem cells, activated β-catenin regulates self-renewal by undergoing translocation to the nucleus, where it interacts with the transcription factors lymphoid enhancer-binding factor 1 (LEF1) and T-cell factor (TCF) to activate the transcription of genes that induce proliferation, such as *Myc* and *Ccnd1*, which encodes cyclin D1[44,45]. Remarkably, a BCR-ABL-dependent increase in MYC protein, but not mRNA levels has been reported to occur in CD34$^+$ progenitors from patients with AP and BC CML, but not patients with CP CML[46].

The finding of β-catenin-mediated self-renewal of GMPs is controversial[47], and further work is required to show whether myeloid transformation originates in this pool in all or only some patients with CML. Nevertheless, the notion that disease progression may be due to the reactivation of self-renewal pathways in a cell population that normally lacks this capacity is of considerable interest[47]. Significantly, activating mutations of β-catenin are found in epithelial cancers, including: adenomatosis polyposis coli (APC)$^{-/-}$ colon carcinoma[48], ovarian tumours[49] and non-small-cell lung cancer[50]. Hence, self-renewal through the activation of β-catenin may prove to be a common mechanism of disease progression in many human tumours.

Genomic instability

The mechanisms for surveying the genome for DNA damage (BOX 1) and repairing these lesions are compromised in CML, and it is likely that similar failures of genome surveillance and DNA repair may contribute to the genomic instability of all human cancers. It has been proposed that BCR-ABL induces mutations in genes responsible for maintaining genomic integrity, and that such mutations function as "amplifiers of a genetically unstable phenotype"[14]. This could explain the occurrence of the non-random chromosomal abnormalities that characterize CML progression. The most frequent are trisomy 8 (33%), an additional Ph chromosome (30%), isochromosome 17 (20%), trisomy 19 (12%), loss of the Y chromosome (8% of males), trisomy 21 (7%) and monosomy 7 (5%)[51]. These changes have been used as markers of disease progression, but may not necessarily be causal agents of transformation. However, it is now clear that these visible cytogenetic abnormalities are markers for genes which, when deleted, mutated, duplicated or otherwise altered, can drive the blastic transformation

Table 1 | **Features of CML evolution and genes implicated in this process**

Feature of disease evolution	Genes implicated	Refs
Differentiation arrest	*CEBPα*	29,31,34,35
	HNRNPE2	38
	NUP98–HOXA9	37
	AML1–EVI1	N/A
Failures of genome surveillance	*ATR*	53,57,63
	BRCA1	61
Deficiencies of DNA repair	*DNA-PKcs*	65–67
	RAD51	70,73,74
	FANCD2	75
	WRN	77
	XPB	80,81
Mutator phenotype	*POLB* (DNA polymerase β)	88,90
	MLH1, PMS2	152
Telomere shortening	*TERT*	96,98,153
Loss of tumour-suppressor function	*PP2A*	100
	TP53	129,130
	CDKN2A	136–138
	INK4A	139
	ARF	145
	RB1	133–135
*Unknown**	*PIASy*	108
	AML1, AF1Q, ETS2, LYL1, PLU1, IMPDH1, GBDR1, NME1, GRO2, CA4, SNC73, MSF, CREBBP	111
	PRAME, GLI2, SOCS2, WT1, GAS2, MDFI, WIT1, RRAS2, ITPR1, FOS, ARG	107
	CD7, PR3, ELA2, BMI1	117,123

*Here we report genes, identified as differentially expressed in advanced or aggressive chronic myeloid leukaemia (CML) through expression profiling, which have not yet been associated with a particular feature of disease evolution.

process. Such changes could be viewed as the tip of the iceberg of an underlying genomic instability and compromised 'genome surveillance' mechanisms that might characterize the advanced stages of CML.

Failure of genome surveillance. A complex surveillance system has evolved in eukaryotes to monitor and respond to genotoxic insults that threaten the integrity of the genome. At its heart are the ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and RAD3-related (ATR) nuclear protein kinases that function as DNA damage 'sensors'.

Although it was once considered a probable scenario to account for disease evolution, the mutation or deletion of *ATM* has been ruled out as a general phenomenon in CML[52]. Rather, it is ATR signalling that is inhibited in CML[53] (FIG. 3a). Despite having a predominantly cytoplasmic localization[12,12,54], BCR-ABL is still able to translocate to the nucleus following exposure to genotoxic agents, such as etoposide. In the nucleus, both ATM and ATR are bound by BCR-ABL, but ATM signalling seems unaffected, as one of its substrates, checkpoint kinase 2 (CHK2) is still phosphorylated[53]. By contrast, ATR is inhibited leading to reduced phosphorylation and activation of its substrate, checkpoint kinase 1 (CHK1). As CHK1 signalling is essential for the activation of the intra-S-phase cell cycle checkpoint (reviewed in REF. 55), this checkpoint is abrogated in CML cells. The inhibition of ATR-signalling by BCR-ABL is expected to cause inappropriate DNA replication[53,56], despite the presence of DNA damage (FIG. 3a). This may account for the radioresistant DNA synthesis phenotype that is a common feature of tumour and leukaemic cells, and has been observed in *BCR-ABL*-transformed cells[53]. Impaired ATR signalling caused by BCR-ABL should predispose leukaemic cells to an increased frequency of deletions and translocations owing to the accumulation of genetic lesions, including DNA double-strand breaks (DSBs). Indeed, these chromosomal abnormalities have been observed by spectral karyotyping in CML cells and etoposide-treated BCR-ABL-expressing cells[57].

Although ATM seems unaffected by its interaction with BCR-ABL, its substrate BRCA1 (REFS 58,59) is profoundly downregulated in CML. The precise function of BRCA1 is unknown, but it has been proposed to act as a scaffold protein within the high molecular weight BRCA1-associated genome surveillance complex (BASC)[60]. Following DNA damage, BRCA1 organizes different damage sensors and coordinates the repair activity. Whereas BRCA1 is highly expressed in mononuclear cells from normal peripheral blood and in cell lines that do not express BCR-ABL, it is virtually undetectable, by immunoblotting, in CML cells and *BCR-ABL*-transformed cell lines[61]. Furthermore, the relationship between BCR-ABL and BRCA1 expression is apparently reciprocal, as shown in a cell line with inducible expression of BCR-ABL[61]. Downregulation of BRCA1 protein levels is dependent on the tyrosine kinase activity of BCR-ABL and occurs through a post-transcriptional mechanism[61]. Although the details of this mechanism remain to be determined, the same authors found that genome surveillance was compromised in BCR-ABL-expressing cells — a higher frequency of ionizing radiation-induced sister chromatid exchange was seen in a cell line transfected with *BCR-ABL* compared with its untransfected control[61]. A downstream substrate of BRCA1 is CHK1 (REF. 62), and the inhibition of CHK1 signalling may prove to be a common theme in the erosion of genome surveillance in BCR-ABL-expressing cells. Recently, however, contradictory findings have been reported in which ATR–CHK1 signalling was found to be stimulated in BCR-ABL-positive cells, with CHK1 being activated by phosphorylation on S345 (REF. 63). This group found no evidence of 'shuttling' of BCR-ABL into the nucleus in response to genotoxic agents, and concluded that BCR-ABL directs the response to DNA damage from the cytoplasm[63]. Further studies should resolve this controversy, but it is clear that BCR-ABL alters normal cellular responses to DNA damage through an interaction with ATR.

Ataxia telangiectasia
A recessive disorder caused by inactivating mutations in the *ATM* gene and characterized by X-ray hypersensitivity, genomic instability and a predisposition to both solid tumours and haematological malignancies.

Intra-S-phase cell-cycle checkpoint
Normally, the activated intra-S-phase checkpoint delays the cell cycle, providing additional time for DNA repair, or for initiating apoptosis, if the damage is irreparable.

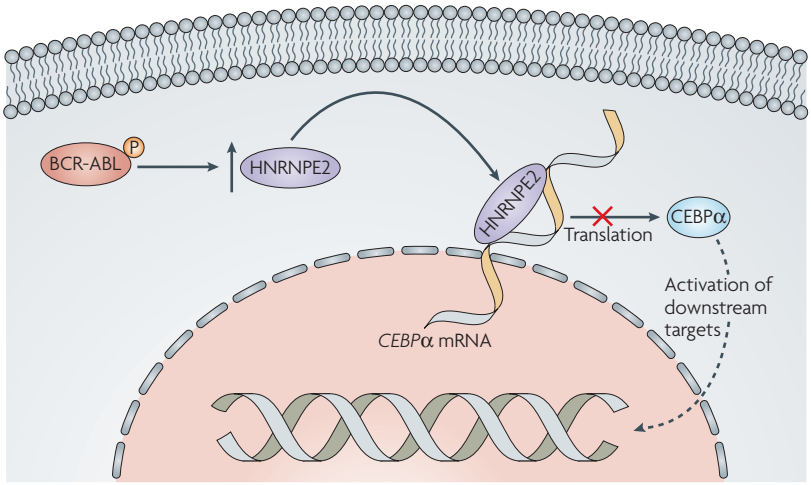

Figure 2 | **BCR-ABL contributes to differentiation arrest through interference with the translation of the transcription factor CEBPα.** BCR-ABL increases the stability of heterogeneous nuclear ribonucleoprotein E2 (HNRNPE2), which binds to a seven nucleotide spacer within the sequence of CEBPα mRNA. The binding of HNRNPE2 to CEBPα mRNA inhibits the subsequent translation of this transcription factor, leading to the transcriptional downregulation of one of its target genes, *CSF3R*, which encodes the granulocyte colony-stimulating factor receptor (GCSFR) required for granulocytic differentiation.

BRCA1-associated genome surveillance complex (BASC). This DNA damage repair complex includes repair proteins and tumour suppressors (MSH2, MSH6, MLH1, ATM and BLM) as well as the RAD50–MRE11–NBS1 complex.

Mononuclear cells The fraction of leukocytes consisting of lymphocytes, monocytes and immature granulocytes (blasts, promyelocytes, myelocytes and metamyelocytes) that is obtained by density centrifugation of the peripheral blood for exclusion of the polymorphonuclear cells (that is, band and segmented granulocytes).

Xeroderma pigmentosum (XP). An inherited genetic disorder characterized by an inability to repair DNA damage resulting from exposure to UV light. Before the discovery of the mutated genes that cause this condition, patients were classified into 7 complementation groups (XPA, XPB, XPC, XPD, XPE, XPF and XPG).

Deficiencies of DNA repair. The repair of DNA DSBs, (reviewed in REF. 64) is problematic, and erroneous joining of free ends of DNA is associated with chromosomal translocations. Mammalian cells repair DSBs by two mechanisms: non-homologous end-joining (NHEJ) and homologous recombination (HR) (BOX 1). Growing evidence indicates that both pathways are faulty in CML. The catalytic subunit of DNA-dependent protein kinase (DNA-PKcs), a key protein in NHEJ, is downregulated in $CD34^+$ CML progenitors but not in normal $CD34^+$ cells[65]. Levels of DNA-PKcs protein inversely correlated with those of BCR-ABL in cell lines, and were almost undetectable in CML cells (FIG. 3b). Suppression of DNA-PKcs was dependent on the tyrosine kinase activity of BCR-ABL, was post-transcriptional and could be reversed with proteasome inhibitors, implying that downregulation was through the ubiquitin–proteasomal pathway[65]. Loss of DNA-PKcs would be expected to have severe consequences for NHEJ; indeed, there is evidence that NHEJ in CML is 'error-prone' or capable of 'mis-repair' when challenged with excess DSBs[66,67]. Apart from CML, deficient NHEJ has been identified in high-grade bladder carcinomas that have a high degree of genomic instability[68], and single nucleotide polymorphisms (SNPs) in the *KU70* and *XRCC4* genes that are also involved in NHEJ are associated with an increased breast cancer risk[69].

The other mechanism for DSB repair, HR, may be abnormally stimulated — to the detriment of its fidelity — in CML. Central to the process of HR is RAD51, and BCR-ABL affects RAD51 expression by regulating its transcription, activation and degradation[70] (FIG. 3c). Transcriptional upregulation of RAD51 involves the activation of the transcription factor STAT5 downstream of BCR-ABL and is dependent on the Src-homology (SH) domains 2 and 3 of BCR-ABL. The activation of RAD51 by BCR-ABL occurs through phosphorylation on T315. Moreover BCR-ABL blocks the proteolytic degradation of RAD51 by preventing the activation of caspase 3 (REFS 71,72). These combined effects of BCR-ABL on RAD51 are manifest as a drug-resistant phenotype in which lesions generated by cytotoxic agents are rapidly repaired by increased HR activity[70]. Although rapid, the BCR-ABL-stimulated HR repairs lack the fidelity of those performed by the HR mechanisms in untransformed cells. Both HR and NHEJ mechanisms have been shown to exhibit a less faithful repair of the DSBs induced by reactive oxygen species (ROS)[73] or γ-irradiation[74] in *BCR-ABL* transformed cells when compared with normal cells. The HR mechanism was found to have an overall mutation rate of 6×10^{-3} in BCR-ABL-expressing cells, of which 55% of the mutations involved either G/C to A/T transitions or G/C to T/A transversions[73]. By contrast, HR was apparently error-free in cells that did not express BCR-ABL[73]. Recently, preliminary reports have suggested that BCR-ABL can also stimulate the expression and activity of other proteins that interact with RAD51, such as Fanconi D2 protein (FANCD2)[75], NBS1 (REF. 76) and the Werner syndrome helicase (WRN)[77]. Collectively, these findings indicate that both the HR and NHEJ pathways become more error-prone in CML through the actions of BCR-ABL, and probably exacerbate genomic instability, contributing to disease progression. Deficient HR seems to be a feature of other tumours, as a recent study found mutations in the partner and localizer of BRCA2 (*PALB2*), in Finnish families with familial breast and prostate cancer[78]. A functional BRCA2–PALB2 complex is essential for HR[78].

DNA damaging agents, such as UV light, can induce 'bulky' or helix-altering lesions, such as cyclobutane pyrimidine dimers and [6-4]photoproducts that are repaired by the nucleotide excision repair (NER) pathway[79]. Deficiencies of NER are responsible for the hereditary condition xeroderma pigmentosum (XP), which predisposes to skin cancer on exposure to UV light. In patients with the group B form of this condition (XPB), a subunit of the TFIIH basal transcription factor, XPB, is mutated, giving rise to the NER-deficient phenotype. In CML, deficiencies in NER might add to the genomic instability that characterizes the advanced phase of the disease. Cell lines of a lymphoid lineage transformed with *BCR-ABL* were twofold less active in their repair of UVC-damaged DNA than the parental cell line[80]. By contrast, BCR-ABL-expressing cell lines of myeloid lineage were twofold more active at repairing UVC-damaged DNA than their parental counterpart[80]. These effects were blocked by imatinib, indicating that a tyrosine kinase-dependent mechanism was involved[80]. At the molecular level, BCR-ABL affects the co-localization of XPB[81] with proliferating cell nuclear antigen (PCNA) through an unknown mechanism[80]. XPB is present in a multi-protein complex, with XPA, XPG and replication protein A (RPA), which is required for the recognition and excision processes of NER[82]. PCNA is a component of a second complex, along with replication factor C (RFC), RPA, DNA polymerase δ and ε

Box 1 | **Sources of DNA damage and repair mechanisms**

Throughout an individual's lifetime the genome sustains DNA damage from three main sources (reviewed in REF. 91): environmental agents, including the ultraviolet (UV) spectrum of sunlight, ionizing radiation and genotoxic chemicals; reactive oxygen species produced during the course of normal oxidative respiration; and damage intrinsic to DNA that results from the labile nature of chemical bonds within the molecule, which undergo spontaneous hydrolysis under physiological conditions leading to the loss of purines (depurination) or amino groups (deamination) from a DNA strand[91]. Cells have evolved different mechanisms to repair DNA damage and prevent mutations.

Non-homologous end joining (NHEJ)
A mechanism for repairing double-strand breaks (DSBs) that has evolved to function in circumstances where the damaged DNA has not yet been replicated to provide a 'template' of homologous sequence for repair. The free ends of the DSB are ligated through the NHEJ pathway, but sequence information is frequently lost as the repair process proceeds without a template. Proteins involved in NHEJ assemble at the free ends of the DSB to form a complex that includes the KU70–KU80 heterodimer, DNA ligase IV, X-ray cross complementation group 4 protein (XRCC4) and the artemis nuclease (reviewed in REF. 127).

Homologous recombination (HR)
The second main DSB repair mechanism is dependent on DNA replication to provide a homologous sequence to that of the damaged strands, which acts as a template for repair. Cells that have progressed through S-phase can use HR to repair chromosomes that have sustained DSBs using sequence information on the sister chromatid as the template.

Nucleotide excision repair (NER)
A mechanism that has evolved for the repair of DNA, which has sustained 'bulky' or helix-altering lesions. In NER, the lesion is recognized and excised within a 25–30 nucleotide sequence that spans it. This is followed by DNA synthesis, which repairs the excised region using the opposite, undamaged strand as a template.

Base excision repair (BER)
A mechanism that has evolved for the repair of point mutations within DNA involving single bases. Mutated bases are 'flipped out' by DNA glycosylases, and the resulting abasic site is filled in by DNA polymerases. DNA polymerases-δ and ε have proofreading activity and are responsible for catalysing most of the DNA polymerization in human cells[128]. DNA polymerase-β has a high error rate of 67×10^{-5} substitutions per incorporated nucleotide, and no exonuclease proofreading activity (reviewed in REF. 128). This compares with error rates estimated as being as low as 1×10^{-5} substitutions per incorporated nucleotide for DNA polymerases δ and ε.

and DNA ligase I, which is essential for the DNA repair synthesis of NER[82]. In addition to increasing genomic instability, alterations in NER may account for why CML cells from advanced phase patients are resistant to cytotoxic drugs that induce DNA lesions similar to those caused by UV irradiation[80]. Deficient NER as a result of the heterozygosity of several of the XP genes has been identified as a risk factor for other tumours, such as familial early-onset lung cancer[83].

Mutator phenotype. The mutator phenotype hypothesis was first proposed by Loeb[84,85] to account for the mutation frequency in malignant cells being higher than the spontaneous mutation rate in normal cells. There is evidence that BCR-ABL can induce a mutator phenotype as mutation rates in an *lacI* reporter gene in a transgenic mouse system were more than twofold higher in the spleen and kidney of *BCR-ABL*-expressing mice than in normal mice[86]. In a subsequent study[87], an increased frequency of insertions and deletions was detected in the tissues of *BCR-ABL*-expressing leukaemic and preleukaemic mice compared with control animals. Furthermore, the mutation rate in the kidneys of *BCR-ABL*-expressing preleukaemic mice could be partially reduced by treating the animals with imatinib[87]. In agreement with these findings, mutation rates at two 'reporter' loci, hypoxanthine guanine phosphoribosyl transferase (HPRT) and Na-K-ATPase, were threefold to fivefold higher in cell lines transformed with *BCR-ABL* than in the parental cells[88]. Recently, the mutagenesis of BCR-ABL has been shown in cells in which the oncoprotein stimulates ROS which, in turn, cause DNA damage resulting in mutations within the oncogene kinase domain[89].

The expression of BCR-ABL was also associated with the upregulated expression of DNA polymerase β[88,90], an enzyme involved in the base excision repair (BER) of abasic sites in DNA[91] (BOX 1). As this specific polymerase has a low-fidelity DNA repair, it might be expected that its increased expression in a leukaemic cell, at the expense of other polymerases with greater accuracy, would promote genomic instability.

Telomere shortening

Telomere shortening occurs in many solid tumours[92] and leukaemias[93]. Moreover, there is an apparent association between telomere shortening in human malignancies and increased genomic instability and disease progression[94]. In CML, the rate of telomere loss increases with disease progression from CP to AP, and when measured in Ph^+ peripheral blood leucocytes was found to be 10–20-fold higher than in normal cells[95]. The extent and rate of shortening was also found to be prognostic for the time to disease progression[95,96]. Paradoxically, telomerase activity has been described as being upregulated in many human cancers, and active telomerase is thought to be essential for the immortalization of malignant cells[97]. This apparent contradiction may be due to the impossibility of obtaining the appropriate 'normal' control for the cancer stem cell in solid tumours[98]. In CML, where such a comparison is possible, the expression of TERT

Depurination
A form of spontaneous DNA damage in which purines (adenine or guanine) are lost from a DNA strand resulting in the formation of an 'abasic' site.

Deamination
Another form of spontaneous DNA damage in which amino groups are lost from the bases adenine, guanine, cytosine or 5-methylcytosine, converting them to their miscoding equivalents of hypoxanthine, xanthine, uracil and thymine, respectively.

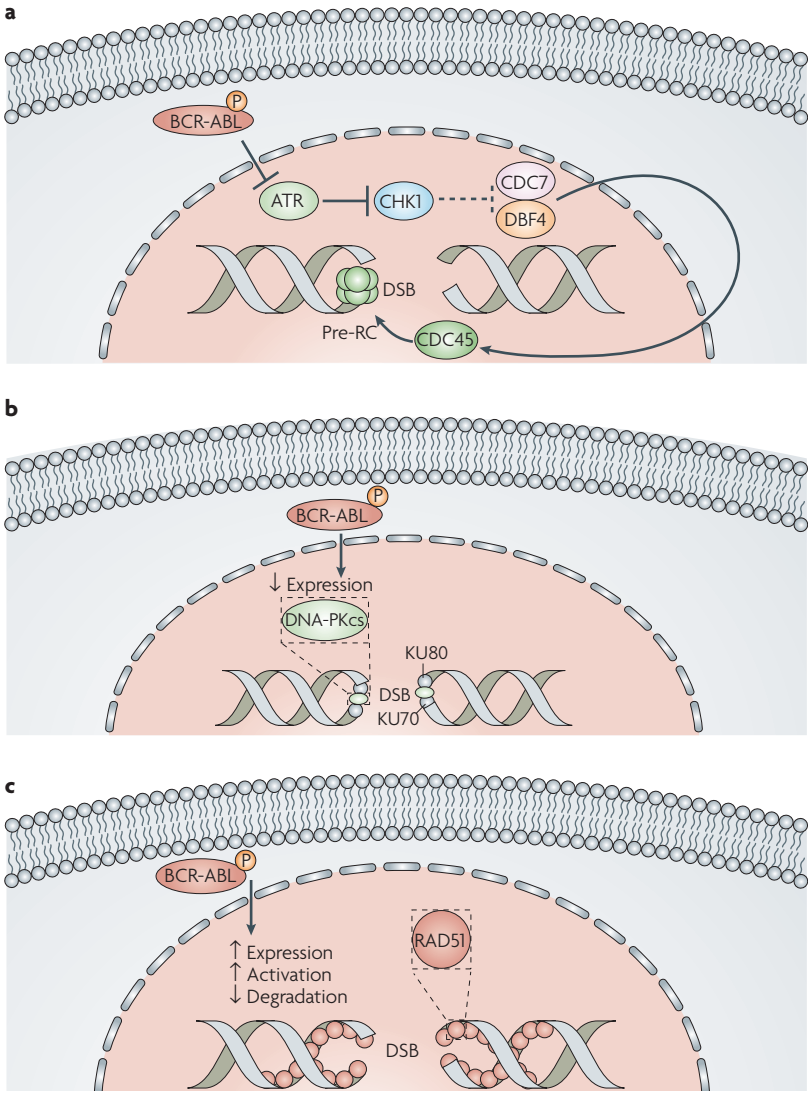

Figure 3 | **BCR-ABL and genomic instability. a** |BCR-ABL inhibits the key genome surveillance kinase ataxia telangiectasia and rad 3-related (ATR). BCR-ABL shuttles to the nucleus, in response to a genotoxic insult, where it binds to and inhibits the DNA-damage sensor kinase — ATR. ATR bound by BCR-ABL fails to catalyse the phosphorylation and activation of its substrate, checkpoint kinase 1 (CHK1), which, in turn, fails to disrupt and inactivate the cell division cycle 7 (CDC7)–DBF4 complex. The active CDC7–DBF4 complex promotes the loading of CDC45 into the pre-replication complex (Pre-RC), allowing inappropriate replication to occur. The interaction between CHK1 and the CDC7–DBF4 complex is indicated with a dotted line, as this is a putative function of CHK1 based on the known role of checkpoint kinases in regulating CDC7 and DBF4 in yeast. This diagram is based on a figure by Dierov *et al.*[53] used to illustrate a model proposed by Costanzo *et al.*[56] **b** | BCR-ABL interferes with the repair of DNA double-strand breaks (DSBs) by inhibiting the expression of the catalytic sub-unit of DNA-dependent protein kinase (DNA-PKcs). DNA-PKcs is downregulated in inverse relation to the expression of BCR-ABL in cells. DNA-PKcs binds to the free ends of DSBs, where it forms a complex with the KU70–KU80 heterodimer. Ligation of the broken strands also requires DNA ligase IV, X-ray cross complementation group 4 protein (XRCC4) and the nuclease artemis (not shown). **c** | BCR-ABL interferes with the repair of DNA double-strand breaks (DSBs) through several effects on the DNA repair protein RAD51. BCR-ABL stimulates the transcription of RAD51 by a mechanism that involves signal transducer and activator of transcription 5 (STAT5). In addition, BCR-ABL activates RAD51 through the phosphorylation of T315 and suppresses its caspase 3-mediated proteolytic degradation (not shown). Homologous recombination (HR) repair of DNA by BCR-ABL-stimulated RAD51 lacks the fidelity of HR repair in untransformed cells. Repair by HR also requires many other proteins, including RAD51 paralogues and the RAD50–MRE11–NBS1 complex (not shown). Part **a** of this figure is modified with permission from REF. 53 © (2004) Elsevier Science.

was found to be significantly lower in CML $CD34^+$ cells than in normal $CD34^+$ cells[98]. This finding is consistent with an accelerating rate of telomere shortening in CML. In addition, wild-type ABL negatively regulates the activity of TERT[99], suggesting that tyrosine kinase inhibitors may be therapeutically useful for slowing telomere loss. Finally, it remains an open question as to whether telomere shortening is a cause of disease evolution in cancer (driving progression by an as yet unknown mechanism), or whether it is merely a symptom caused by the increased turnover of malignant cells.

Loss of tumour-suppressor function

CML is an example of a neoplasm in which tumorigenesis is driven by a potent oncogene, *BCR-ABL*, and the importance of inactivated tumour-suppressor genes is less clear. Nevertheless, BCR-ABL is a tyrosine kinase, so it follows that enzymes with the opposite function (protein phosphatases) but sharing the same substrates are likely to function as tumour suppressors. This observation is likely to hold true for other kinases that transduce an oncogenic signal. Consequently, lessons learned from tumour-suppressor phosphatases in CML might be applicable to, or have relevance for, other cancers. Indeed the protein phosphatase 2A (PP2A) acts as a tumour suppressor in CML by antagonizing BCR-ABL[100] (FIG. 4). The inactivation of PP2A is associated with disease progression because, whereas the activity of this phosphatase is only moderately impaired in $CD34^+$ cells from CML CP patients, its activity in CML BC cells is negligible. BCR-ABL was found to inhibit PP2A by post-transcriptional upregulation of SET, a phosphoprotein that functions as a physiological inhibitor of PP2A[100]. The effect of BCR-ABL on SET expression was dose-dependent, and correlated with the increased expression of BCR-ABL, which is a feature of advanced phase disease in CML[22,24–27]. It is likely that BCR-ABL exploits a common mechanism for inactivating tumour-suppressor phosphatases, as SET is frequently overexpressed in solid tumours and leukaemias[100]. Significantly, BCR-ABL and PP2A were found to share protein substrates, including MAPK, STAT5 and Akt. It is possible that the expression of active PP2A is

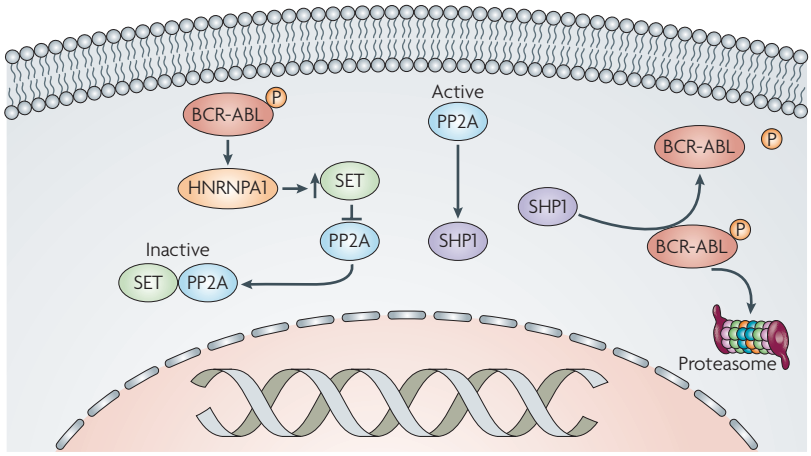

Figure 4 | **BCR-ABL inactivates the tumour suppressor protein phosphatase 2A (PP2A).** BCR-ABL inhibits PP2A by post-transcriptional upregulation of SET, a phosphoprotein that is a physiological inhibitor of PP2A[100]. The effect of BCR-ABL on SET expression is mediated by the heterogeneous nuclear ribonucleoprotein A1 (HNRNPA1). SET is frequently overexpressed in leukaemias and solid tumours. It is likely that the expression of active PP2A is incompatible with disease progression in chronic myeloid leukaemia, as BCR-ABL itself is a target of this phosphatase through SHP1 (Src-homology region 2 domain-containing protein tyrosine phosphatase 1), which catalyses the dephosphorylation of BCR-ABL[100]. This, in turn, promotes the downregulation of BCR-ABL through proteasomal degradation.

incompatible with disease progression in CML, as this phosphatase recruits and activates the Src homology region 2 domain-containing protein tyrosine phosphatase 1 (SHP1), which catalyses the dephosphorylation of BCR-ABL[100]. This, in turn, promotes the downregulation of BCR-ABL through proteasomal degradation[100]. Consistent with this mechanism, the silencing of SET by small interfering RNA (siRNA), the ectopic overexpression of PP2A or its pharmacological stimulation with forskolin, all led to reduced expression of BCR-ABL[100]. In view of the mutual antagonism of BCR-ABL and PP2A, the inhibition of PP2A may be a prerequisite for disease progression in CML. In particular, increasing expression of BCR-ABL associated with disease progression[22,24–27] would be expected to cause further stimulation of SET expression leading to the inhibition of the available pool of PP2A[100].

Although not a universal requirement for blastic transformation, abnormalities of other tumour-suppressor genes are associated with disease progression in subgroups of patients with CML (BOX 2).

Rubinstein–Taybi malformation syndrome
An inherited condition characterized by short stature, broad thumbs and first toes, unusual facial features and moderate to severe mental retardation. Individuals with this condition are predisposed to developing non-malignant and malignant tumours, as well as having an increased risk of developing leukaemia or lymphoma. The condition is caused by inactivating autosomal dominant mutations of the *CREBBP* gene, which is involved in regulating cell division and growth.

Profiling disease-progression gene candidates

There have been major changes in the management of patients with CML over the past 5–10 years. Imatinib, the first ABL kinase inhibitor developed for clinical use, is now the most common first-line therapy[101] (BOX 3), with allogeneic stem cell transplantation usually being reserved for a small proportion of selected young patients who have a histologically identical donor[102]. For patients who develop resistance to imatinib, the new second-generation kinase inhibitors such as nilotinib and dasatinib offer a further opportunity to control the disease[103,104]. However, each of these therapeutic strategies has its own limitations, represented mainly by a high rate of morbidity and mortality in the case of patients who undergo bone marrow transplants[102], and the development of resistance[105] and persistence of residual disease in the case of the existing tyrosine kinase inhibitors[106]. Furthermore, none of the currently available treatments have a significant impact on the control or reversal of advanced disease, making it mandatory to aim at curing CML while it is still in CP. It is therefore vital to identify good prognostic markers of disease progression in CML to identify those patients who are most likely to benefit from a particular treatment option. Unfortunately, most current clinical and molecular tests are unable to provide this information, and it is therefore not possible to tailor therapies according to the risks faced by each patient. This has been likened to being unable to predict where on the 'clock' of disease progression an individual lies at the time of diagnosis[107]. However, with the advent of microarray technology there is a realistic chance of identifying suitable biomarkers of altered gene expression.

Gene-expression profiling studies have identified candidate genes, which are differentially regulated in CML BC relative to CP samples, that might be responsible for disease progression. The first study found that the protein inhibitor of activated STAT (*pIASy*) was downregulated in CML BC[108]. This was an interesting candidate because BCR-ABL induces the phosphorylation and activation of STAT proteins[109,110], and reduced expression of this inhibitor might be expected to potentiate STAT signalling. Another investigation[111] identified 13 genes that were consistently deregulated by fourfold or more in all CML samples analysed, and were therefore thought to be relevant for malignant transformation. These were genes encoding transcription factors *AML1*, *AF1Q*, *ETS2* and *LYL1*, a chromatin binding protein (*PLU1*), an enzyme involved in nucleotide metabolism (inosine-5-monophosphate dehydrogenase), genes associated with other malignancies (*GBDR1*, *NME1*, *GRO2*, *CA4*, *SNC73* and *MSF*) and a gene that causes Rubinstein–Taybi malformation syndrome upon inactivation (*CREBBP*). These previous reports used small sample sizes, only 13 (REF. 108) and 10 (REF. 111) patients with CML, respectively. More recently, a gene-expression profile was reported based on 91 cases of CML (42 CP, 17 AP and 32 BC)[107]. Overall, an expression signature was obtained suggesting that disease evolution in CML is a two rather than a three-stage process, as the expression of genes in AP strongly correlated with their levels in BC[107]. Notably, deregulation of the *WNT*–β-catenin pathway, reduced expression of the transcription factors JUNB and FOS, upregulation of the *ABL*-related gene (*ARG*, also known as *ABL2*) and increased expression of *PRAME* (preferentially expressed antigen of melanoma) were associated with disease progression[107]. As described previously, activation of the *WNT*–β-catenin pathway has been observed in GMP from patients with CML[43], where it was associated with self-renewal. The concomitant downregulation of JUNB and deregulation of *WNT*–β-catenin in their gene-expression profile led these authors to propose that these pathways may be linked by the *MDF1* gene, an inhibitor of basic helix-loop-helix transcription factors[107]. They propose that β-catenin-mediated gene activation may be altered by changes to *MDF1* and

Box 2 | **Other tumour-suppressor genes implicated in blast crisis**

Inactivating mutations or deletions of the tumour-suppressor gene *TP53* are found in up to 30% of cases of blast crisis (BC) (none with a lymphoid phenotype, most being myeloid and a minority with a mixed phenotype), but not chronic phase (CP) chronic myeloid leukaemia (CML)[129,130]. Furthermore, in those patients with CML in myeloid BC without *TP53* mutations, the functional loss of p53 might be the consequence of increased protein degradation owing to post-translational[131] and/or translational[132] BCR-ABL-dependent promotion of its inhibitor MDM2. Reports vary as to the frequency with which the retinoblastoma 1 (*RB1*) gene is mutated or deleted in CML[133–135], but a recent estimate indicates a proportion of 20% of CML BC cases[51]. Homozygous deletion of the *CDKN2A* locus occurs in a significant percentage (29%–50%; estimates vary) of cases of CML BC of lymphoid, but not myeloid, phenotype[136–138]. This locus encodes two tumour-suppressor proteins, INK4A (REF. 139) and ARF (REF. 140). The INK4A protein binds to the cyclin-dependent kinases CDK4 and CDK6, thereby preventing their association with D cyclins and p21 (reviewed in REF. 141). This, in turn, increases the availability of p21, which inhibits cyclin E–CDK2 and cyclin A–CDK2-mediated hyperphosphorylation of RB1. In its hypophosphorylated state, RB1 binds to the E2F transcription factor causing a G1 cell-cycle arrest[142]. Somatic loss of INK4A, through mutation or deletion, has been reported in thousands of human cancers (reviewed in REF. 143). The ARF protein stabilizes p53 in the nucleus by preventing its MDM2-mediated cytoplasmic export and degradation[144]. Loss-of-function mutations affecting ARF are not as widespread in human cancers as those affecting INK4A, but they have been described in colon cancer, familial melanoma and astrocytoma[143]. There has been the suggestion that the *CDKN2A* locus may be transcriptionally silenced by promoter methylation in advanced phase CML of lymphoid origin[145], although this has been disputed[138]. However, the reasons why abnormalities of individual tumour-suppressor genes preferentially affect specific lineages of CML are not currently known.

its interaction with the β-catenin-binding protein axin[107]. ARG is a cytoplasmic protein involved in cell signalling, although its targets have yet to be identified, and *PRAME* is a nuclear protein that acts as a dominant repressor of retinoic acid receptor (RAR) signalling[107].

CML is especially amenable to gene-expression profiling of disease progression because of its clearly-defined clinical phases. However, even in other human neoplasias in which such distinctions are less clear, microarray analysis has proven valuable in identifying genes associated with disease progression[112–116].

Yong *et al.* used a new approach for studying disease progression in CML[117]. They used oligonucleotide microarrays to identify genes differentially regulated between $CD34^+$ progenitors from patients with 'indolent' disease (more than 7 years before the onset of BC) and from patients whose disease followed an 'aggressive' course (onset of BC within 3 years of diagnosis)[117]. As the heterogeneity of disease progression is a common feature of many human cancers, including CML, efforts to determine whether it has a genetic basis are crucial to determine the prognosis and to establish the best therapeutic strategies. Two main explanations for the different rates of progression may be envisaged. Either patients with CML have an equal chance of their disease progressing to BC, or this chance is not equal. In the former case, disease progression would be stochastic, and patients would have no genetic predisposition or susceptibility favouring more rapid transformation. The heterogeneity of CP duration could be explained as being due to the timing of the appearance of deleterious mutations in essential genes. In the latter case, differences in gene expression at the presentation of CP would determine the individual susceptibility to disease progression. The heterogeneity of CP duration reflects the heterogeneity in patterns of genes expressed by different patients. The findings of Yong *et al.* would tend to support the second hypothesis, as 20 genes were identified as having a pattern of expression that was significantly different between patients with aggressive and indolent disease[117]. Multivariate analysis showed that the expression of some genes correlated with patient survival. In particular, the combination of low expression of *CD7* with high expression of proteinase 3 (*PR3*) or elastase (*ELA2*) was found to be highly predictive of longer survival[117]. CD7 is a differentiation marker that is expressed on T cells and immature myeloid progenitors, and its high expression has also been associated with poor prognosis in AML and myelodysplastic syndrome (MDS)[118,119]. PR3 and ELA2 are serine proteases that accumulate in the primary azurophilic granules of granulocytes, and are overexpressed in CML compared with normal progenitors[120,121]. As both proteins are targets of cytotoxic T lymphocytes in CML[120,122], it is possible that in patients with overexpression of these two antigens in primitive haematopoietic cells, the resultant immune-mediated cytoprotection renders their CP relatively indolent, thereby reducing the risk of progression to BC. An alternative model would be that in patients with greater numbers of $CD34^+CD7^+$ cells at diagnosis, the t(9;22) translocation occurred in a more primitive stem cell than that of patients who had smaller numbers of $CD34^+CD7^+$ cells. If such a differential acquisition of the *BCR-ABL* gene results in a phenotypic difference in the course of CML, with an inferior survival as a consequence of the more immature $CD34^+CD7^+$ stem cell, this would be consistent with the poor prognosis generally associated with the most undifferentiated subtypes in many malignancies. In CML, the presence of *BCR-ABL* in a stem cell population with increased self-renewal capacity would result in a higher risk of random mutation and, consequently, of a mutation in a gene capable of inducing a more malignant phenotype occurring early in the evolution of CP, leading more rapidly to BC. This is supported by recent findings that the expression of the polycomb group gene *BMI1*, which regulates the proliferation of both normal and leukaemic stem cells, is significantly higher in patients with advanced phase than in patients with CP CML[123]. Of note, the level of *BMI1* at diagnosis correlated with time of transformation to BC, and the combination of low *BMI1* and high *PR3* expression was associated in multivariate analysis with an improved overall survival.

To date, the genes identified in microarray studies as being associated with advanced phase disease in CML have not been implicated in disease progression in other human tumours. However, it is the identification of pathways that contain differentially regulated genes that may be of greater value, as these may be involved in the disease evolution of several types of malignancies. For example, the *WNT*–β-catenin pathway, which has already been discussed with regard to CML, has been reported to be activated in colon carcinoma[124]. Furthermore, a significant overlap of transcriptionally deregulated *WNT*–β-catenin

Multivariate analysis
The application of a statistical method for the simultaneous analysis of more than two variables.

Primary azurophilic granules
Lysosomes found in the cytoplasm of granulocytes that store enzymes, essential for catalysing the 'respiratory burst' of neutrophils that is directed against pathogens or infected cells. The lysosomes are known as 'azurophilic' granules as they are readily stained with azure dyes.

Box 3 | **Imatinib and disease progression**

One of the most remarkable and intriguing results of the treatment of chronic myeloid leukaemia (CML) with imatinib is the low rate of progression to advanced phase or blast crisis (BC) (2% at 5 years follow-up) in patients who achieve and maintain a complete cytogenetic response within the first 12–18 months after starting the drug treatment[101]. The biological basis of this phenomenon is unknown, but is a matter of great interest and speculation. As BCR-ABL increases the level of genomic instability and unfaithful DNA repair by the various mechanisms here discussed, continuous inhibition of its kinase activity by imatinib should lead to a decreased risk of mutations in general, including in genes that can trigger the blast crisis process. Moreover, although imatinib may be unable to kill the leukaemic stem cell, it may drastically reduce its rate of proliferation and self-renewal, driving it to a deep and prolonged quiescence where the chances of DNA breaks and mis-repair are lower in the absence of DNA replication. Another possible mechanism relies on the evidence that the cell of origin of BC may not be a 'true' stem cell, but rather a more committed granulocyte-macrophage progenitor (GMP)[43,146], and it has been shown that imatinib can eliminate a large proportion of these cells[147–149], reducing the population at risk of blastic transformation. An additional aspect of the 5-year clinical trial follow-up study was the observation that the rate of disease progression is not only low, but seems to decrease with time under successful treatment, and the reasons for this are not entirely clear. As the emergence of a sub-clone of leukaemic cells with mutations in the kinase domain of BCR-ABL is the main cause of relapse in patients treated with imatinib, it is reasonable to suppose that the chances of this happening at any time during treatment depend largely on the size of the mutant sub-clone at the start of therapy and on its proliferation rate. Therefore, assuming that both the original (non-mutated) and the mutant BCR-ABL clones have a similar doubling time[150,151], it could be predicted that the highest risk for a mutant clone to become dominant and lead to relapse and disease progression occurs within the first years of therapy. A longer follow-up of chronic phase patients treated up-front with imatinib should confirm whether such a trend for a continuous decrease in the risk of disease evolution is statistically significant.

signalling genes was found between colon and rectal carcinomas, suggesting that similar pathways are involved in carcinogenesis[124].

It seems likely that microarray gene-expression profiling will continue to identify many candidate genes. The next challenge is obviously the validation of the findings in large and independent series of patients to show that such genes are functionally important for the development of BC.

Conclusions and future directions

Disease progression in cancer is complex and multi-factorial. In CML, no single cytogenetic or molecular genetic event can be responsible for the heterogeneous nature of blastic transformation. It remains an open question whether many secondary mutations are needed for the transformation to BC to occur. This number is unlikely to be the same for all patients, and some cases of BC are probably precipitated by fewer mutations than in others because these mutations are in more crucial genes.

Differentiation arrest is a common feature of tumours or leukaemia with a poor prognosis. A mechanism for the differentiation arrest of CML progenitors has been proposed to involve the inhibition of CEBPα, highlighting the importance of epigenetic mechanisms in cancer progression.

Although the cause of the impaired genome surveillance of CML cells remains controversial, BCR-ABL has been shown to affect ATR–CHK1 signalling. Moreover, in CML, deficiencies in most of the major repair pathways have been found, including BER, NER, NHEJ and HR.

Some of the mechanisms involved in disease progression in CML could potentially be exploited for therapeutic advantage. It has been proposed that agents that prevent the interaction of PRAME with RAR are likely to be effective in advanced phase CML, as the forced overexpression of PRAME prevents RAR-mediated differentiation, growth arrest and apoptosis[107]. More generally, where genomic instability is associated with *BRCA1* deficiency, it has been suggested that the inhibition of the DNA repair enzyme poly (ADP-ribose) polymerase-1 (PARP1) could be used to generate specific lesions that require BRCA1 for their repair (reviewed in REF. 125). As BRCA1 is deficient, other more error-prone mechanisms are forced to act, resulting in chromosome abnormalities that trigger a loss of viability — a strategy known as 'synthetic lethality'[125]. So far, the best developed translational idea based on our knowledge of genes that are predominantly altered in BC of CML, is the targeting of the *PP2A* tumour-suppressor gene. Restoration of PP2A activity with FTY720, a synthetic analogue of sphingosine-1-phosphate, promotes apoptosis and impairs the *in vitro* clonogenicity of cells from patients with BC CML, and suppresses leukaemogenesis in immunodeficient mice transplanted with *BCR-ABL*-transformed myeloid and lymphoid progenitors[126]. As FTY720 has been shown to be well tolerated in phase I–III clinical trials for multiple sclerosis or solid organ transplant patients, these findings strongly support its use as a novel therapeutic approach for BC CML.

The identification of biomarkers of disease progression may help to identify novel therapeutic targets, which can be exploited in patients who have developed resistance to existing therapies. These are exciting times for oncology, in which advances in technology coupled with a 'critical mass' of knowledge will lead to rapid advances in our understanding of the mechanisms that underlie neoplastic progression.

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Competing interests statement
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DATABASES

The following terms in this article are linked online to:
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ABL | *AF1Q* | *AML1* | APC | ATM | ATR | *BCR* | *BMI1* | BRCA1 | BRCA2 | β-catenin | *CA4* | caspase 3 | *CCND1* | *CD7* | CD34 | CEBPα | CHK1 | CHK2 | *CREBBP* | *ELA2* | *ETS2* | *EVI1* | FANCD2 | FOS | *GBDR1* | *GCSFR* | *GRO2* | HNRNPE2 | *HOXA9* | HPRT | *ID1* | JUNB | *KU70* | LEF1 | *LYL1* | *MYC* | *MSF* | NBS1 | *NME1* | *NUP98* | *PALB2* | PARP1 | PCNA | *pIASy* | *PR3* | RAD51 | RFC | RPA | SET | SHP1 | STAT5 | TERT | TFIIH | WRN | XPA | XPG | *XRCC4*
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