REVIEW

Signal transduction therapy in haematological malignancies: identification and targeting of tyrosine kinases

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ABSTRACT

Tyrosine kinases play key roles in cell proliferation, survival and differentiation. Their aberrant activation, caused either by the formation of fusion genes by chromosome translocation or by intragenic changes, such as point mutations or internal duplications, is of major importance in the development of many haematological malignancies. An understanding of the mechanisms by which BCR-ABL contributes to the pathogenesis of chronic myeloid leukaemia led to the development of imatinib, the first of several tyrosine kinase inhibitors to enter clinical trials. Although the development of resistance has been problematic, particularly in aggressive disease, the development of novel inhibitors and combination with other forms of therapy shows promise.

INTRODUCTION

TKs (tyrosine kinases) play key roles in major cellular processes, such as proliferation, survival and differentiation. They were among the first oncogenes to be discovered in the 1970s and, since then, there has been steady progress in understanding their normal structure and function and how disturbances of these can contribute to leukaemia. It is now known that TKs, or components of their signalling pathways, are mutated in many forms of leukaemia and that leukaemic cells may be dependent on aberrant TK signalling for their survival. The rational design of small molecule inhibitors of TK signalling has therefore been an area of active research for several decades and resulted in the development of imatinib (Gleevec, also known as STI571 or Glivec), the first such molecule to enter clinical trials for the treatment of CML (chronic myeloid leukaemia). Clinical trials showed imatinib to be highly effective with respect to inducing cytogenetic and haematological remission, and imatinib is now the first-line treatment of choice for patients with CML. Other inhibitors of ABL and other kinases are now either in clinical trials or in development.

This review surveys the role of TKs in leukaemia and targeting these abnormalities with selective signal transduction inhibitors, with particular focus on BCR-ABL and other MPDs (myeloproliferative disorders).

STRUCTURE AND FUNCTION OF TKs

TKs catalyse the transfer of γ-phosphate from ATP to the hydroxy group of tyrosine residues both within the

Key words: BCR-ABL, haematological malignancy, imatinib, leukaemia, signalling inhibitor, tyrosine kinase.

Abbreviations: ALK, anaplastic lymphoma kinase; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CEL, chronic eosinophilic leukaemia; CML, chronic myeloid leukaemia; CMML, chronic myelomonocytic leukaemia; EGFR, epidermal growth factor receptor; EMS, Eight p11 myeloproliferative syndrome; EPO, erythropoietin; FGFR, fibroblast growth factor receptor; FIP1L1, Fipt1-like 1; FLT3, Fms-like tyrosine kinase 3; FISH, fluorescence in situ hybridization; HES, hypereosinophilic syndrome; IFNo, interferon α; IL3, interleukin 3; IRIS, International Randomized study of Interferon and STI571; ITD, internal tandem duplication; JAK, Janus kinase; MDS, myelodysplastic syndrome; MPD, myeloproliferative disorder; NTRK, neurotrophin receptor kinase; PDGFR, platelet-derived growth factor receptor; Ph, Philadelphia chromosome; PV, polycythaemia vera; RT, reverse transcription; SCT, stem cell transplantation; TK, tyrosine kinase.

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kinase itself (autophosphorylation) or other proteins in downstream signalling pathways. Autophosphorylation generally serves either of two purposes. First, the phosphorylated tyrosine can cause a change in the tertiary structure of the protein by becoming a site for intramolecular bond formation or by altering charge distribution. In general, changes such as this switch on the kinase activity by inducing an ‘active’ conformation of the protein. Secondly, autophosphorylated tyrosine residues can serve as docking sites for other molecules involved in signal transduction.

Of the 90 human TKs, about two-thirds are cell-surface receptors with extracellular ligand-binding domains, a hydrophobic transmembrane domain and an intracellular TK catalytic domain. An intracellular juxtamembrane region is known to play an important inhibitory role in many receptors. In general, binding of the ligand to the receptor induces receptor dimerization, resulting in partial activation of the kinase domain. Phosphorylation of key tyrosine residues then promotes further structural alteration of the kinase to a fully active conformation, allowing further autophosphorylation and phosphorylation of protein substrates (Figures 1a–1c). Activated receptors are generally internalized by endocytosis, resulting in their down-regulation and degradation. There is some evidence that receptors can continue signalling from endosomes [1], and some kinases, e.g. FGFRs (fibroblast growth factor receptors) and EGFRs (epidermal growth factor receptors), can also be translocated to the nucleus and regulate transcription [2,3].

Many of the non-receptor TKs, such as those of the JAK, SRC and ZAP70/SYK gene families, form part of complexes involved in signalling from receptors that do not themselves have intrinsic TK activity, such as cytokine, T-cell and Ig receptors. As with receptor TKs, non-receptor TKs have auto-inhibitory domains that stabilize the protein in a non-active conformation.

ONCOGENIC TK MUTATIONS

TK mutations can be divided into two types: first, those where intragenic point mutations, deletions, insertions or internal duplications serve to activate the kinase in a constitutive manner, and secondly, those in which a novel fusion gene is created by chromosomal rearrangement. Although amplification and overexpression of certain TKs may be oncopgenic in model systems and is believed to play a role in the genesis of some epithelial tumours, there is little evidence to suggest that this is important in haematological malignancies.

Intragenic mutations activate TKs principally by three mechanisms: (i) by mutations in the extracellular domain of a receptor TK that promote receptor dimerization, (ii) by mutation of an inhibitory domain, e.g. juxtamembrane mutations in type III TK receptors and the

(a) Inactive kinase (b) Partially active kinase

(c) Fully active kinase (d) Kinase bound to an inhibitor

Figure 1 Mechanism of activation for a generalized TK

(a) The N- and C-lobes of the kinase domain of an inactive receptor TK are indicated with activation loop in red. (b) Phosphorylation of tyrosine residues (PY) within inhibitory regions (not shown) and the activation loop results in partial kinase activation and a conformational change of the activation loop. (c) Full kinase activation follows with further autophosphorylation and phosphorylation of substrate molecules. (d) Binding of an ATP-competitive inhibitor, such as imatinib, prevents kinase phosphorylation and activation.

JAK2 (Janus kinase 2) V617F (Val$^{617}$ → Phe) mutation (see below), and (iii) by activating mutations of the TK domain e.g. KIT D816V (Asp$^{816}$ → Val) mutation (see below).

Fusion genes are largely formed by cytogenetically visible chromosomal translocations. In a small number of cases, fusion genes are the result of submicroscopic chromosome deletions or rearrangements. In all cases known to date, the N-terminal region of a partner gene is fused in-frame to the C-terminal region of the kinase, including the catalytic domain. The partner gene provides a promoter to drive transcription of the fusion in haemopoietic progenitor cells and, in nearly all cases, an oligomerization domain that is essential for kinase activation. The paradigm TK fusion gene is BCR-ABL, which is considered in more detail below. Table 1 lists the principal mechanisms and examples of TK activation in haematological malignancy.

BCR-ABL: a paradigm for TK fusion genes

The BCR-ABL fusion gene is formed as a result of the chromosomal translocation t(9;22)(q34;q11) and is seen
in essentially all patients with CML and approx. 25% of adult patients with ALL (acute lymphoblastic leukaemia). The translocation breakpoints nearly always fall within introns 14 or 15 of the BCR gene and within the first intron of the ABL gene. Thus the first 13 or 14 exons of BCR are fused to exon 2 through to the C-terminus of ABL. The BCR portion controls transcription of the fusion gene and provides an oligomerization domain, whereas the ABL portion retains its full TK catalytic domain. Oligomerization of the fusion protein therefore results in activation and autophosphorylation of the ABL kinase domain, leading to elevated levels of signalling through proliferative and anti-apoptotic pathways such as RAS/MAPK (mitogen-activated protein kinase), STAT (signal transducer and activator of transcription) and PI3K (phosphoinositide 3-kinase)/Akt [4,5].

There is good evidence that aberrant signalling from BCR-ABL is the principal and probably sole cause of chronic phase CML. If murine bone-marrow cells are transfected with BCR-ABL and transplanted back into mice, the recipients develop a MPD closely resembling CML [6]. In addition, introduction of BCR-ABL into the IL3 (interleukin 3)-dependent murine cell line Ba/F3 renders it IL3-independent [7]. Cells from CML patients are therefore largely thought to be dependent on aberrant BCR-ABL signalling for their survival, and this notion provides the rationale for the inhibitory activity of imatinib and other inhibitors.

### DEVELOPMENT OF TK INHIBITORS

In the 1970s and 1980s, a number of compounds were discovered that appeared to inhibit the activity of protein TKs. For example, the antibiotics geldanamycin [8] and herbimycin A [8,9] and the protease inhibitor Tos-Lys-CH2Cl (tosyl-lysylchloromethane; 'TLCK') [10] converted Rous-sarcoma-virus-infected rat kidney cells into a normal morphology, apparently by inhibiting the activity of the SRC TK. Herbimycin was shown to inhibit the transforming activity of a number of TKs, including ABL, but not the non-TKs RAS, RAF or MYC [11]. Although these compounds were eventually found not to inhibit TK catalytic activity directly, this work established the idea that TK inhibition had therapeutic potential.

A series of compounds called tyrphostins, derived from the actinomycete-derived EGFR inhibitor erbstatin, which compete for the tyrosine substrate rather than ATP, were among the first to be systematically developed to inhibit TKs [12]. Some tyrphostins had activity towards ABL and critically were shown to have higher affinity for the fusion protein BCR-ABL than normal ABL [13]; however, none of these compounds were developed for clinical use. Imatinib (Gleevec, also known as STI571 or Glivec), the first TK inhibitor to enter clinical trials, was one of a series of compounds belonging to the 2-phenylaminopyrimidine class developed by Ciba Geigy, which inhibits TK activity by targeting the ATP-binding pocket, rather than that of the substrate, and also gains specificity by recognizing an inactive conformation of the activation loop [14] (Figure 1d). In addition to BCR-ABL, imatinib also inhibits ABL2 (ARG), PDGFRα (platelet-derived growth factor receptor α; encoded by PDGFRα) and PDGFRβ (platelet-derived growth factor receptor β; encoded by PDGFRβ), KIT and CSF1R (colony-stimulating factor 1 receptor)/FMS [15–17]. The use of imatinib in disorders with mutations of these kinases is discussed in the relevant sections below.

One requirement for the development of TK inhibitors has been cell-based systems for measuring the activity of potential inhibitory compounds. Cell lines derived from patient material carrying known TK mutations are particularly useful. Many such lines exist in the case of BCR-ABL and have been important tools in inhibitor studies [18,19]. However, for many mutated kinases, e.g. FGFR1 and PDGFRB fusion genes, such cell lines do not exist. An alternative has been the use of IL3-dependent lines, most commonly the murine Ba/F3 pro-B-cells [20]. The introduction of an activated TK into Ba/F3 cells renders them IL3-independent and, in medium lacking IL3, dependent on the mutated TK for growth and/or survival. The ability of a TK mutation to induce IL3-independent growth/survival is therefore a test of the transforming potential of the mutation, and IL3-independent cell lines thus created can be used to test the activity of inhibitory compounds. The dose required to inhibit cell growth by 50% (IC50) is a widely used standard measurement of inhibition.

TK inhibitors in development or in clinical trials and their known targets are listed in Table 2. Considering that these compounds typically inhibit a number of kinases, rather than being completely specific for a single kinase, and the fact that they inhibit both normal and
Table 2 TK inhibitors

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>TK targets</th>
<th>References</th>
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<tbody>
<tr>
<td>Imatinib*</td>
<td>ABL, ABL2, KIT, PDGFR, PDGFRβ and CSF1R</td>
<td>[19,21,22]</td>
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<tr>
<td>AMN107*</td>
<td>ABL, KIT and PDGFR</td>
<td>[23–27]</td>
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<td>Dasatinib (BMS-354825)*</td>
<td>ABL, SRC kinases, KIT D816 and PDGFR</td>
<td>[28–30]</td>
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<tr>
<td>OND12380†</td>
<td>ABL</td>
<td>[31]</td>
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<tr>
<td>VX-680*</td>
<td>Aurora kinase and ABL</td>
<td>[32]</td>
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<tr>
<td>AP23464</td>
<td>ABL, SRC and KIT D816</td>
<td>[33,34]</td>
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<td>AP23848</td>
<td>ABL, SR and KIT D816</td>
<td>[33,35]</td>
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<tr>
<td>PPI</td>
<td>ABL, SRC and KIT D816</td>
<td>[36,37]</td>
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<tr>
<td>CGP76030</td>
<td>ABL and SRC</td>
<td>[37]</td>
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<td>SKI-606*</td>
<td>ABL and SRC</td>
<td>[38]</td>
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<tr>
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<td>ABL and SRC</td>
<td>[39]</td>
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<td>ABL</td>
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<td>P0180970</td>
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<td>CHIR-259*</td>
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<td>FL3</td>
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<td>PDGFR, VEGFR1/2, KIT and FLT3</td>
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<td>VEGFR, PDGFR, KIT and FLT3</td>
<td>[50,51]</td>
</tr>
<tr>
<td>SUS416* (Semaxanib)*</td>
<td>FLT3, VEGFR2 and KIT</td>
<td>[52]</td>
</tr>
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<td>MLN518*</td>
<td>KIT D816</td>
<td>[41,53]</td>
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<tr>
<td>JZ23819</td>
<td>FLT3</td>
<td>[54]</td>
</tr>
<tr>
<td>GNF-2†</td>
<td>BCR-ABL</td>
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* Compounds in clinical trials (not necessarily for haematological malignancies).† Compounds with targets other than the ATP-binding site.

mutant kinases, it is remarkable that these inhibitors are generally well tolerated.

A SURVEY OF TK MUTATIONS AND INHIBITORS

ABL

BCR-ABL and CML

Initial in vitro and in vivo studies established the effectiveness of imatinib in inhibiting the activity of BCR-ABL in cell-free and cell-based systems, by tumour formation in mice and in colony assays of primary cells from CML patients [19,21,22].

Phase I trials demonstrated that imatinib was highly effective in inducing haematological and cytogenetic remission in CML chronic phase patients who had failed therapy with IFNα (interferon α) [56] and also induced responses, albeit generally of short duration, in CML blast crisis and Ph (Philadelphia chromosome)-positive ALL [57]. In 2002, phase II trials confirmed these preliminary results in IFNα-resistant late chronic phase [58], accelerated phase [59] and blast crisis [60] with complete haematological responses in 95 %, 34 % and 8 %, major cytogenetic responses in 60 %, 24 % and 16 % and complete cytogenetic responses in 41 %, 17 % and 7 % respectively.

The phase III IRIS (International Randomized study of Interferon and STI571) trial, which compared imatinib with combination therapy of IFNα and cytarabine, was complicated by high rates of crossovers and discontinuations due to the obvious therapeutic advantages of imatinib and eventual approval by the U.S. Food and Drug Agency. However, the study was still able to demonstrate the clear superiority of imatinib with estimated 18-month complete cytogenetic response rates of 76.2 % for imatinib compared with 14.5 % for IFNα/cytarabine [61]. A recent IRIS trial update with an average duration of 38 months of imatinib treatment focused on imatinib patients, since very few patients remained on IFNα and cytarabine [62]. Complete haematological and cytogenetic remissions were 96 % and 81 % respectively, whereas 94 % of patients remained free of progression to accelerated phase or blast crisis. Overall estimated survival at 42 months was 91 %.

Imatinib resistance and the development of novel compounds

The dramatic successes described here have been tempered by the appearance of imatinib resistance which occurs at a rate of approx. 2 % of cases per annum for patients treated in chronic phase, but much more frequently for patients treated in accelerated phase or blast crisis. Three classes of disease resistance have been identified: (i) an increase in expression of BCR-ABL, usually by gene amplification [63], (ii) BCR-ABL-independent resistance, e.g. by up-regulation of SRC-related kinases [64], and (iii) by point mutations which interfere with imatinib binding [65,66].

Several areas of research are being explored with the aim of preventing or overcoming resistance. These include immunotherapy [67], the use of inhibitors such as farnesyl transferase inhibitors to inhibit molecules that lie downstream of BCR-ABL signalling [68], and the development of novel TK inhibitors which are active against imatinib-resistant BCR-ABL mutants.

Like imatinib, most novel TK inhibitors that inhibit BCR-ABL compete with ATP. Two such inhibitors in clinical trials are AMN107 [25] and the dual ABL/SRC inhibitor dasatinib (BMS-354825) [30]. Both are orders of magnitude more active against BCR-ABL than imatinib, with IC50 values in the low nanomolar range. In addition, these compounds are active against all of the imatinib-resistant mutations tested with the exception of T315I (Thr315 → Ile). Thr315, referred to as the 'gatekeeper', lies within the ATP-binding site and is conserved in many kinases. Mutations of homologous threonine residues in the KIT (Thr670) [69] and EGFRs (Thr790) [70] also result in resistance to imatinib in gastrointestinal stromal tumours and to gefitinib in lung cancer respectively. In phase I trials, both AMN107 and dasatinib have induced
significant responses in CML patients who failed imatinib therapy [71–73]. Other compounds targeted at the ATP-binding site with activity towards ABL (but with very variable specificity) are AP23464 and AP23848 [34], PP1 [36,37], CGP76030 [37], SKI-606 [38], NS-187 (CNS-9) [39] and PD166326 [40]. A search among compounds with established targets has also identified the ATP-competitive compound VX-680, an inhibitor of aurora kinase, as having activity against T315I [32].

A strategy to circumvent resistance to compounds that target the ATP-binding site has been to identify inhibitors that compete for substrate rather than ATP binding, or which bind other allosteric sites. ON012380 [31] is competitive for the kinase substrate and is active against all tested kinase-domain-resistance mutations, including T315I, with an IC_{50} of approx. 10 nmol/l. A novel class of compound, GNF-2, has recently been developed that appears to be specific for BCR-ABL with no detected off-target activity including towards normal ABL [55]. Although there is some uncertainty about its mechanism of action, GNF-2 appears to bind the myristoyl pocket and thereby stabilizes BCR-ABL in an inactive state.

Imatinib resistance and the development of novel therapies have recently been reviewed in detail [65,66,74–76]. No doubt the concepts and strategies developed in relation to imatinib resistance will also be relevant to the targeting of other TK mutations.

**BCR-ABL-positive ALL**

The **BCR-ABL** fusion gene is seen in approx. 25 % of adult ALL patients and is associated with a particularly poor prognosis. Long-term survival rates for patients treated with chemotherapy alone is less than 10 %. SCT (stem-cell transplantation) undertaken during the first complete remission is the treatment of choice with long term survival of 30–60 %; however, this is rarely successful in patients who fail initial therapy.

Initial trials in patients with relapsed or refractory disease demonstrated that imatinib as a monotherapy could induce remission in some patients, but these were generally short lived [77]. More recent trials have used imatinib in combination with chemotherapy as consolidation therapy after achieving complete remission before SCT. Trials have shown substantially improved disease or event-free and overall survivals of at least 2-fold compared with historical data [78–80]. A study of the use of imatinib following chemotherapy in elderly patients has shown a dramatic improvement in relapse and survival with projected 1-year overall survival, relapse-free survival and event-free survival rates of 71 %, 58 % and 57 % respectively, compared with historical data of 43 %, 11 % and 5 % respectively [81]. As with CML, the development of imatinib resistance is of major significance in determining relapse, and the novel inhibitors dasatinib and AMN107 are currently in clinical trials for Ph-positive ALL [82–84].

**Other rare ABL translocations**

To date, three rare variant ABL fusions have been identified that involve ETV6, EML1 or NUP214. ETV6-ABL has been described in 14 cases with atypical CML, ALL or AML (acute myeloid leukaemia). Of the ten cases with cytogenetic data, three showed an apparent t(9;12)(q34p13), two were complex involving a third chromosome and five showed involvement of only 9q34 or 12p13. Three patients were treated with imatinib and had responses: one patient with CML blast crisis had a response of short duration [85], a second CML patient in chronic phase after chemotherapy for blast crisis achieved a complete haematological and partial cytogenetic remission for 92 days [86], and a third CML patient treated in chronic phase remained in complete cytogenetic remission at 1 year [87]. Cryopreserved cells from a fourth patient also showed an in vitro response to imatinib [88].

A single case of EML1-ABL, formed as a consequence of a cytogenetically cryptic t(9;14)(q34q32), was identified in a screen of 116 patients with T-ALL (T-cell ALL) [89]. EML1-ABL transformed Ba/F3 cells to IL3-independence, and growth of these cells was inhibited by imatinib at similar levels to that needed to inhibit BCR-ABL.

The **NUP214-ABL** fusion was identified initially in five out of 85 (5.8 %) cases of patients with T-ALL as a consequence of a novel mechanism of fusion gene formation: a small intrachromosomal translocation and production of episomes [90]. The episomes are formed by apparent excision and religation of a stretch of DNA containing 3′-ABL and 5′-NUP214 (which lies approx. 200 kb telomeric of ABL at chromosome band q34). The **NUP214-ABL** fusion gene is formed at the site of religation. Extrachromosomal amplification of ABL, presumably **NUP214-ABL**, has also been described in five out of 210 (2.3 %) children with T-ALL, three out of 70 (4.3 %) adults with T-ALL [91] and two out of 30 adult T-ALL cases in a third study [92]. A second mechanism of formation of the fusion gene, described in a single patient with T-ALL, involved insertion and amplification of **NUP214-ABL** into the long arm of chromosome 2 [93].

Treatment of three **NUP214-ABL**-positive cell lines, PEER, BE-13 and ALL-SIL, with imatinib demonstrated a reduction in phosphorylation of **NUP214-ABL** and Cskl [90], although imatinib only inhibited proliferation of one of these lines. Imatinib therapy has been reported in one patient with T-ALL who achieved complete remission with conventional treatment, but relapsed while on maintenance therapy [94]. After failing to respond to maintenance chemotherapy, imatinib was initiated but without response. It is possible that **NUP214-ABL** amplification may mirror genomic amplification of BCR-ABL, a known mechanism of imatinib resistance in Ph-positive ALL, and that higher imatinib doses might be more successful in **NUP214-ABL** patients.
In conclusion, imatinib has greatly improved the prospects for patients with chronic phase CML and treatment with the second generation of inhibitors is likely to improve this further. The high rate of therapy resistance that accompanies CML blast crisis, however, remains highly problematic. It remains to be seen whether targeted therapy will improve the poor prognosis of acute leukaemia patients with ABL translocations.

**PDGFRA**

**FIP1L1-PDGFRα**

Following the successful treatment of CML patients, imatinib was trialled in other conditions. Remarkably, several patients with idiopathic HES (hypereosinophilic syndrome) had rapid and clear responses, suggesting that the disease was being driven by an imatinib-responsive TK [95,96]. All the responders had a normal karyotype and thus there were no clues as to the identity of the putative kinase, but a responsive patient was later identified who carried a t(1;4)(q44;q12). Since the imatinib-responsive gene **PDGFRA** lies at 4q12, FISH (fluorescence in situ hybridization) and RACE (rapid amplification of cDNA ends)-PCR studies were used to confirm that **PDGFRA** was rearranged by the translocation and to identify a translocation partner gene. Surprisingly the partner gene turned out to be the **FIP1L1** gene (encoding FIP1-like 1), which also lies at 4q12, approx. 800 kb upstream of **PDGFRA**. In other words, the translocation was the visible component of a complex rearrangement and was not directly relevant to the formation of the **FIP1L1-PDGFRα** fusion, although it served a critical role in identifying that abnormality [97]. The **FIP1L1-PDGFRα** fusion gene was subsequently identified in nine out of 16 patients with HES as a result of a cytogenetically invisible interstitial deletion. **FIP1L1-PDGFRα** was also independently identified by proteomic techniques in the imatinib-responsive EOL1 cell line [98].

The phenotype of **FIP1L1-PDGFRα**-positive disease was studied in a series of 89 patients with eosinophilia. Eleven cases (14%) were **FIP1L1-PDGFRα**-positive, all were male and, in addition to eosinophilia, all but one patient had a characteristic mast cell infiltration of the bone marrow forming loose ill-defined aggregates that were only clearly visible by tryptase staining [99]. However, the precise phenotype and classification of **FIP1L1-PDGFRα**-positive disease remains a matter of debate [100,101]. The biology of **FIP1L1-PDGFRα** has recently been reviewed in detail [102,103]. Although the first study suggested that the frequency of **FIP1L1-PDGFRα** in cases with an initial diagnoses of HES was high (>50%), our own experience and that of others [103] suggests that, among unselected patients with HES, a figure of 10–15% is more realistic.

Imatinib is now generally considered to be the treatment of choice for **FIP1L1-PDGFRα**-positive patients. In a recent follow-up report of 11 cases treated for a median of 24 months (range, 1–40 months), all remained in complete haematological remission [103]; in another study, many patients achieved molecular remission [104]. Although the development of imatinib resistance appears to be infrequent, two additional inhibitors, AMN107 [27] and PKC412 [105], have shown activity against **FIP1L1-PDGFRα** with IC50 values of <23 and 130 nmol/l respectively. In addition, a novel tricyclic pyrazole compound JNJ-10198409 [106] shows considerable *in vitro* activity against PDGFRs (IC50 = 4 nmol/l), and other known PDGFR inhibitors, such as dasatinib, are also likely to be effective against **FIP1L1-PDGFRα**.

**BCR-PDGFRα**

A translocation of **PDGFRA** with **BCR** has been reported in four patients [107–109] with a CML-like disease with eosinophilia and t(4;22)(q12;q11). It is possible that some **BCR-PDGFRα** patients have been misdiagnosed as Ph-positive CML, since the t(4;22) could be misinterpreted as a simple variant of the Ph translocation. Two of the patients were treated with imatinib and had complete haematological responses [108,109].

**PDGFRB**

Translocations involving **PDGFRB** are rare, but well documented. A total of nine partner genes have been described so far, most of which have only been identified in single cases. The most frequently occurring translocation is the t(5;12)(q31;p13) which gives rise to the **ETV6-PDGFRβ** fusion gene [110] and has been described in aCML (atypical CML), AML, CMML (chronic myelomonocytic leukaemia), CEL (chronic eosinophilic leukaemia) and unclassified MPDs, usually in association with peripheral blood or marrow eosinophilia [111]. Other partner genes are **CEV14** [112], **HIP1** [113,114], **H4** [115–117], **RABEP1** [118], **PDE4DIP** [119], **KIAA1509** [120], **TPS3BP1** [121], **HCMOGT-1** [122] and **NIN** [123], which occur in a similar range of diseases.

Imatinib is a potent inhibitor of **PDGFRβ** with an IC50 value of 150 nmol/l [124]. Several cases of **ETV6-PDGFRβ** [125,126] and single cases of **KIAA1509-PDGFRβ**, **PDE4DIP-PDGFRβ**, **NIN-PDGFRβ** and **RABEP1-PDGFRβ** have been treated successfully with imatinib. All had complete haematological and cytogenetic remission, with the longest follow up of 18 months [120]. Two patients (with **ETV6-PDGFRβ** and **RABEP1-PDGFRβ**) achieved molecular remission. One patient with aCML in accelerated phase and an **H4-PDGFRβ** fusion failed to respond to imatinib [127], and a patient with **TPS3BP1-PDGFRβ** relapsed with resistant disease [121].

AMN107 is active against **ETV6-PDGFRβ** with an IC50 value of 18.1 nmol/l [27], and is also active.
KIT mutations in systemic mastocytosis

The transforming oncogene v-kit was first identified in a feline sarcoma retrovirus [130] and its cellular homologue was identified a year later [131]. KIT is essential for the development of erythrocytes, mast cells, germ cells, melanocytes and the intestinal interstitial cells of Cajal and has been implicated in human malignancies, particularly gastrointestinal stromal tumours [132], mast cell malignancies, AML and also some cases of testicular germ cell tumours [133]. The first human oncogenic mutations of KIT were identified in the cell line HMC-1 [134], which harbours two activating mutations V560G (Val560 → Gly; within the juxtamembrane region) and D816V (within the kinase domain). The D816V mutation was later identified in the majority of patients with systemic mastocytosis [135–137]. Rare mutations have also been described near to Asp816 and in the juxtamembrane region [136,138,139]. Several families with mastocytosis have been described with mutations in the extracellular [140] or juxtamembrane regions [141,142], but not in the kinase domain.

Although wild-type KIT is imatinib-sensitive, the Asp816 change hinders imatinib binding, resulting in imatinib-insensitivity. Imatinib is therefore unsuitable for treatment of Asp816-positive patients. However, several compounds have been shown to inhibit D816V in Ba/F3 assays: PKC412 with an IC50 value of 30–40 nmol/l in one study [143] and 100–300 nmol/l in a second study [23], AMN107 with IC50 values of 0.5–3 µmol/l [23,144], MLN518 with an IC50 value of 250 nmol/l [41], and dasatinib with an IC50 value of 200–250 nmol/l [28]. Dasatinib has also shown efficacy in the high nanomolar range in an in vitro mast cell viability assay using primary cells from D816V-positive patients [145]. A screen of 14 different KIT mutations for their ability to transform Ba/F3 cells and their sensitivity to imatinib and PKC412 also demonstrated the high levels of sensitivity of all mutations to both compounds, with the exception of the Asp816 mutation and imatinib [146].

The use of the HMC-1 cell line in inhibitor assays has clearly demonstrated the differential inhibition of Asp816 and other mutations. Variant HMC1.1 carries the juxtamembrane V560G mutation alone, whereas HMC1.2 carries both V560G and D816V. Imatinib, dasatinib and AMN107 inhibit HMC1.1 with IC50 values of 1–10 nmol/l, 1–10 nmol/l and 3–10 nmol/l respectively, whereas HMC1.2 is inhibited with IC50 values >10 µmol/l, 50–100 nmol/l and 1–5 µmol/l respectively [23,28]. PKC412 inhibits both variants with IC50 = 50–250 nmol/l [23].

A single case report describes treatment of a patient with mast cell leukaemia with PKC412, resulting in a partial response with improvements in liver function and significant reductions in peripheral blood mast cells and KIT D816V frequency. However, the disease progressed to AML and the patient died after 3 months of therapy [147].

KIT mutations in AML

In AML, several types of mutation have been identified: deletions and insertions involving Asp199 in exon 8 encoding a portion of the extracellular domain [148] and missense mutations, primarily of codons Asp816 and Asn622 within the kinase domain, are most commonly seen, whereas ITDs (internal tandem duplications) [149] are rare. KIT mutations are found more frequently in patients with the core-binding factor translocations t(8;21) and inv(16) and occur in approx. 40–50% of these patients [150–152]. A number of different substitutions are seen for Asp816, including D861V, D816Y [153] and D816H [154]. In both adult and paediatric t(8;21) leukaemias, the presence of KIT mutations has been associated with a substantially higher relapse and worse survival [150,152,155,156].

A phase II pilot study of imatinib in patients with AML expressing KIT but without mutations resulted in complete or partial responses in five out of 21 patients [157], whereas another study of 36 KIT-expression-positive patients with AML (but with no information about KIT mutation) reported no significant responses [158].

Significant responses to KIT inhibitors have been reported in individual AML cases [159–162], including two patients with exon 8 mutations, one who had a complete remission with imatinib and mild chemotherapy at second relapse [160], and a second who had disappearance of the KIT mutation, but no haematological response [159].

FGFR1

FGFR1 translocations form a rare but distinct group of haematological malignancies. The most common translocation is t(8;13)(p11;q11) in which the ZNF198-FGFR1 fusion gene is formed [163–166]. Other translocation partners are BCR [167–169], TIF1 [170], MYO1A [171], CEP110 [172], FGFR1OP1 (also known as FOP) [173,174], FGFR1OP2 [175] and HERV-K [176]. Most cases show a distinctive MPD, usually with eosinophilia and often associated with a B-cell or, more commonly, T-cell lymphoma termed EMS (Eight p11 myeloproliferative syndrome). An exception is patients with BCR-FGFR1, who develop a disease resembling BCR-ABL-positive CML. EMS is aggressive
with transformation to acute leukaemia in most cases within 1 or 2 years after diagnosis.

In vitro studies have shown that PKC412 inhibits the growth of ZNF198-FGFR1-transformed Ba/F3 cells at concentrations of approx. 200 nmol/l, and a single patient with progressing ZNF198-FGFR1-associated disease demonstrated a partial response to this compound [48]. SU5402 and PD173074 have also been shown to inhibit the growth of ZNF198-FGFR1-transformed cells [177].

**FGFR3**

Approx. 15 % of cases of multiple myeloma have the t(4;14)(p16;q32) translocation which in most cases, although not all, results in deregulation of FGFR3 expression by juxtaposition with IGH regulatory sequences. Rarely, patients with the t(4;14) also harbour an activating FGFR3 K650E (Lys650 → Glu) mutation [178]. In addition, a single case has been reported of a patient with T-cell lymphoma and a t(4;12)(p16p13) translocation, resulting in an ETV6-FGFR3 gene fusion [179].

Several lines of evidence have demonstrated sensitivity of cells with mutated or deregulated FGFR3 to inhibition with FGFR inhibitors. Cell lines containing the t(4;14) translocation are sensitive to the inhibitors SU5402 [177,180], PD173074 [177], PKC412 [181] and CHIR-258 [43], and both Ba/F3 cells and murine models containing FGFR3 K650E and ET6v-FGFR3 show responses to PKC412 [181]. CHIR-258 was also effective in a xenograft mouse model of myeloma [43].

**FLT3**

FLT3 (Fms-like TK3) is predominantly expressed by haemoPoietic progenitor cells and is involved in the control of progenitor cell proliferation and differentiation. In leukaemia, FLT3 is highly expressed by the majority of patients with AML and B-ALL (B-cell ALL) and a minority of patients with T-ALL.

Alterations to FLT3 are among the most common activating mutations in AML and are seen in approx. 30 % of patients. Mutations are of three types: in-frame ITD of the juxtamembrane region (encoded by exons 14–15, formerly referred to as exons 11–12) of varying length in approx. 25 % of cases [182–187], missense mutations of the TK domain predominantly at Asp835, but less frequently at other nearby codons in approx. 7 % [188,189], and, rarely, missense mutations within the juxtamembrane domain [190,191]. FLT3 mutations have been associated with a poor prognosis [183,184], and a recent study of a large number of patients has restricted the poor prognosis to ITDs alone [192].

A number of FLT3 inhibitors have reached phase I or II trials, including SU11248 [50,193], SU5416 [52], CEP-701 [45], CHIR-258 [194] and PKC412 [195]. A novel inhibitor Ki23819 [54] has not reached clinical trials, but has shown high activity towards FLT3 in vitro. In trials, the inhibitors were generally well tolerated and, in a small number of cases, patients have had haematological responses lasting up to several months. However, responses were generally of short duration and trials of FLT3 inhibitors combined with chemotherapy are ongoing [196,197].

Recently FLT3 mutations have also been seen in specific subtypes of ALL. Mutations of the TK domain have been described in 15–18 % of infants with MLL-rearranged ALL [198,199]. TK-domain mutations and juxtamembrane deletions have been described in 21–25 % of patients with paediatric hyperdiploid ALL [198,200], and ITDs and TK-domain mutations have been seen in 3–4 % of adult and paediatric cases of T-ALL [201,202].

**JAK2**

**JAK2 V617F**

The discovery of the acquired activating JAK2 V617F mutation in a wide range of MPDs has been one of the most important haematological discoveries of recent years. The mutation is found in approx. 80 % of patients with PV (polycythaemia vera), 40 % of patients with idiopathic myelofibrosis and 30 % of patients with essential thrombocythaemia [203–207]. The mutation has also been found in CNL (chronic neutrophilic leukaemia; approx. 20 %) [208], CML-like and unclassified MPDs (approx. 20 %) [209,210], MDS (myelodysplastic syndrome; approx. 1–5 %) [205,208,210] and CMML (approx. 3–13 %) [208,210], but not in CML and normal cells. Both in vitro transformation assays and mouse models have demonstrated the transforming activity of the mutation. Mice transplanted with bone marrow transfected with JAK2 V617F developed erythrocytosis [203], and expression of JAK2 V617F in Ba/F3 cells containing the EPO (erythropoietin) receptor conferred EPO hypersensitivity and EPO-independent survival [205].

At present there are no inhibitors of JAK2 in clinical trials, and the development of inhibitors for clinical use may be problematic because of the biological pleiotropism of the JAK family members and their high degree of homology. Consequently, it may be difficult to generate inhibitors that do not give rise to undesirable side effects. Nevertheless, preliminary proof-of-principle experiments demonstrating inhibition of a JAK2 V617F-positive cell line have been described [205], and there is likely to be considerable interest in signal transduction therapy for MPDs in the coming months and years. Several JAK3 inhibitors have already been developed as potential immunosuppressants for transplantation procedures [211].

Although JAK2 V617F is not thought to be imatinib-responsive, approx. 75 % of patients with PV treated with imatinib had either partial or complete haematological responses [212,213]. A study of the levels of V617F in patients with PV on imatinib treatment showed that
patients with a partial or no response had, on average, a slight increase in the level of V617F, whereas the two patients with a complete response had a 2–3-fold reduction in V617F levels [214]. It is likely that the responses in these cases are due to inhibition of a kinase, perhaps KIT, required for erythropoiesis, rather than inhibition of JAK2 or possibly another pathogenic TK.

**JAK2 translocations**

Three translocations that target JAK2 have been identified: t(9;12)(p24;p13), t(9;22)(p24;q11) and t(8;9)(p22;p24), resulting in the ETV6-JAK2 [215,216], BCR-JAK2 [217] and PCM1-JAK2 [218–221] fusion genes respectively. The BCR-JAK2 fusion has been described in a single patient with typical CML, ETV6-JAK2 in T- and B-ALL and aCML, and PCM1-JAK2 in mostly aCML, but also AML, pre-B-ALL, CEL, erythroid leukaemia, MDS/MPD and T-cell lymphoma. It is likely that inhibitors developed for JAK2 V617F would also be active against JAK2 translocations.

**ALK (anaplastic lymphoma kinase)**

The t(2;5)(p23;q35) translocation, which fuses the ALK TK to nucleophosmin (NPM-ALK), is characteristic of anaplastic large cell lymphoma, a distinct clinical entity with an undifferentiated T-cell morphology and expression of CD30 (Ki-1). The NPM-ALK fusion is seen in approx. 80% of ALK-positive anaplastic large cell lymphoma, whereas in the remainder ALK is fused with other partner genes, including TPM3, TFG, ATIC, CLTC, MSN, TMP4, ALO17 (KIAA1618) and MYH9 (for a recent review, see [222]). A fusion of ALK with CLTC has also been described in diffuse large B-cell lymphomas [223,224]. Surprisingly, a number of ALK fusions, although not NPM-ALK, are also seen in inflammatory myofibroblastic tumours [225–229]. As ALK is not normally expressed in T-cells, ALK overexpression as a consequence of gene fusion may be conveniently detected by immunochemistry [230].

Although there are no ALK inhibitors available for clinical use, a recent in vitro study has shown selective inhibition of Ba/F3 NPM-ALK cells and inhibition of proliferation and induction of apoptosis in NPM-ALK-positive cell lines using fused pyrrolcarbazole-derived compounds [231], thereby demonstrating that targeted therapy may be a viable therapeutic option for ALK-positive tumours.

**Other rare TK translocations**

A number of TK fusion genes have been identified only in single cases or in cell lines. A t(6;12)(q21;p13) translocation gave rise to an ETV6-FRK fusion gene in a single case of AML-M4 [232], and LCK has been found to be up-regulated by juxtaposition to TCRB (encoding T-cell receptor β) in a patient with T-ALL (plus a cell line derived from the patient, SUP-T12) and a second cell line HSB-2 [233]. ETV6-ABL2 fusion genes have been identified in material from a patient with AML-M4 [234] and in two cell lines derived from patients with AML [235] and T-ALL [236]. Two SYK fusions have been described: ITK-SYK, resulting from a t(5;9)(q33;q22) translocation in five out of 30 unclassified peripheral T-cell lymphomas [237], and a single case of ETV6-SYK, resulting from a t(9;12)(q22;p12) translocation, in MDS [238]. Finally, a single case of ETV6-NTRK3, resulting from a t(12;15)(p13;q25) translocation, has been described in AML [239]. The ETV6-NTRK3 fusion is also seen in congenital infantile fibrosarcoma [240], congenital mesoblastic nephroma [241] and secretory breast carcinoma [242]. Whether other mutations of these genes will be found to be more common in leukaemia remains to be seen.

Inhibitors of several of these TKs are either in use or in development. ABL2 is inhibited by imatinib, and SRC kinases would be expected to be sensitive to the dual SRC/ABL kinase inhibitors being developed for CML resistance, including dasatinib. There is interest in the development of NTRK3 (neurotrophin receptor kinase 3) inhibitors for treatment of paediatric tumours [243], and SYK inhibitors to control inflammatory response [244]. SYK may also be a useful therapeutic target in mantle cell lymphomas, since SYK amplification and overexpression has been seen in this disease [245].

**DETECTION OF ABNORMALITIES OF TKs**

Detection of TK abnormalities in haematological malignancies requires a range of techniques, most commonly standard cytogenetics, FISH, reverse transcription (RT)–PCR, genomic DNA PCR, sequencing and other point-mutation-detection techniques. Currently there is no diagnostic role for whole-genome-scanning techniques, such as gene expression profiling or microarray comparative genomic hybridization, in the identification of mutant TKs.

Standard cytogenetic analysis will detect most of the chromosome rearrangements that give rise to the currently known TK fusion genes. In MPDs, translocations with breakpoints at 9q34, 4q12 and 5q31-33 should always be investigated for rearrangements of ABL, PDGFRα and PDGFRβ respectively, since patients with these fusion genes have shown excellent responses to imatinib. Detection of the FIP1L1-PDGFRA fusion gene is more problematic, since the chromosome rearrangement is cytogenetically invisible. However, we suggest that all patients with persistent unexplained eosinophilia or a BCR-ABL-negative MPD with eosinophilia should be screened for the FIP1L1-PDGFRA fusion by RT-PCR and/or by FISH for CHIC2 deletion. It is important to note that no other cytogenetically cryptic imatinib-responsive rearrangements have been described thus far.
in MPDs and, therefore, cytogenetic analysis remains the most important front line test for these abnormalities. In addition, translocations in MPD patients involving 8p11 are likely to involve FGFR1. Although the development of FGFR inhibitors is not as advanced as that for ABL, two potential inhibitors of these fusions, PKC412 and CHIR-258, are currently in clinical trials for AML.

A number of inhibitors are in trials or in development that are active against the KIT D816V mutation, although there is little published experience of their use in patients. Detection of the mutation in patients with systemic mastocytosis is not always straightforward, since the proportion of mutant cells is often low. Bone marrow, rather than blood, is the tissue of choice, and methods to increase the sensitivity of detection of D816V have recently been described, but not yet fully evaluated [246].

In acute leukaemia, the prognostic implications of specific TK mutations is increasingly guiding treatment, e.g. post-remission stratification depending on BCR-ABL status, among other factors, has become standard practice in the treatment of ALL. Although the success of TK inhibitors in acute leukaemia patients with mutations of ABL, FLT3 and KIT has been very limited, current trials combining inhibitors with standard chemotherapy or new inhibitor combinations may overcome these hurdles.

In general, for all haematological malignancies it is likely that, in addition to indicating prognosis for subsets of patients, the presence of TK mutations will increasingly contribute to genetic-based molecular classifications of disease entities and determine specific targeted signal transduction therapies. However, it remains to be seen if mutations of TKs or their downstream signalling components will be discovered in most haematological malignancies or whether these abnormalities are largely restricted to those described above.

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