BJP British Journal of Pharmacology

DOI:10.1111/bph.12183
www.brjpharmacol.org

Theme Section: Emerging Therapeutic Aspects in Oncology

REVIEW

Targeting survival pathways in chronic myeloid leukaemia stem cells

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Keywords
chronic myeloid leukaemia (CML); BCR-ABL; tyrosine kinase inhibitors (TKI); haemopoietic stem cell (HSC); imatinib (IM); survival; leukaemic stem cell (LSC); pathways; drugs.

Received
14 November 2012
Revised
11 February 2013
Accepted
19 February 2013

Chronic myeloid leukaemia (CML) is a clonal myeloproliferative disorder characterized by the presence of a fusion oncogene BCR-ABL, which encodes a protein with constitutive TK activity. The implementation of tyrosine kinase inhibitors (TKIs) marked a major advance in CML therapy; however, there are problems with current treatment. For example, relapse occurs when these drugs are discontinued in the majority of patients who have achieved a complete molecular response on TKI and these agents are less effective in patients with mutations in the BCR-ABL kinase domain. Importantly, TKI can effectively target proliferating mature cells, but do not eradicate quiescent leukaemic stem cells (LSCs), therefore allowing disease persistence despite treatment. It is essential that alternative strategies are used to target the LSC population. BCR-ABL activation is responsible for the modulation of different signalling pathways, which allows the LSC fraction to evade cell death. Several pathways have been shown to be modulated by BCR-ABL, including PI3K/AKT/mTOR, JAK-STAT and autophagy signalling pathways. Targeting components of these survival pathways, alone or in combination with TKI, therefore represents an attractive potential therapeutic approach for targeting the LSC. However, many pathways are also active in normal stem cells. Therefore, potential targets must be validated to effectively eradicate CML stem cells while sparing normal counterparts. This review summarizes the main pathways modulated in CML stem cells, the recent developments and the use of novel drugs to target components in these pathways which may be used to target the LSC population.

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Abbreviations
ALOX5, arachidonate 5-lipoxygenase; AP, accelerated phase; As_2O_3, arsenic trioxide; As_4S_4, arsenic sulphide; BC, blast crisis; CML, chronic myeloid leukaemia; CMR, complete molecular response; CP, chronic phase; CQ, chloroquine diphosphate; CyPG, cyclopentenone prostaglandin; DAS, dasatinib; EPA, eicosapentaenoic acid; FOXO, forkhead O transcription factors; G-CSF, granulocyte-colony stimulating factor; GSK3, glycogen synthase kinase 3; HCQ, hydroxychloroquine; HDAC, histone deacetylase; HDACI, histone deacetylase inhibitors; Hh, hedgehog; hnRNPs, heterogeneous nuclear ribonucleoproteins; HSC, haemopoietic stem cell; IM, imatinib mesylate; LSC, leukaemic stem cell; LT-CIC, long-term culture initiating cells; MCyR, major cytogenetic response; MMR, major molecular response; NIL, nilotinib; PML, promyelocytic leukaemia; PON, ponatinib; PP2A, protein phosphatase 2A; PUFA, polyunsaturated fatty acids; SAHA, suberoylanilide hydroxamic acid; SIP, sphingosine 1 phosphate; SK1, sphingosine kinase 1; SMO, smoothened; TKI, tyrosine kinase inhibitors

Introduction

Chronic myeloid leukaemia (CML) is a clonal myeloproliferative disorder characterized by the presence of BCR-ABL, which is an oncogene created by the fusion of *BCR* and *ABL* genes (Rowley, 1973). The juxtaposition of these genes in response to genetic mutation encodes a novel fusion gene that translates into a protein with constitutive TK activity. This deregulated activity present in the haemopoietic stem cell (HSC) population results in the pathogenicity of the disease with the overproduction of mature myeloid cells in the bone marrow and peripheral circulation. The disease is

characterized by three distinct phases; beginning in chronic phase (CP), developing into accelerated (AP) and then blast crisis (BC) phases, which are progressively more aggressive (Sawyers, 1999).

Over the last decade, TK inhibitors (TKIs) were introduced as a revolutionary treatment against the activity of the oncoprotein. TKI, imatinib mesylate (IM; Glivec©, Novartis Pharmaceuticals, Camberley, Surrey, UK), is currently used as the standard treatment in patients with newly diagnosed CP CML. The drug functions through binding to the kinase domain of BCR-ABL and inhibits the activity of the kinase domain through stabilizing the protein in an inactive conformation (Druker *et al.*, 1996). Although the use of IM has been shown to work exceptionally well as a therapy against CML, the drug cannot cure patients of the disease. The French Stop Imatinib (STIM) trial reported that 60% of patients who had achieved and maintained a complete molecular response (CMR) for 2 years on IM relapsed at a median of 4 months upon cessation of their TKI (Mahon *et al.*, 2010). At the other end of the spectrum, there remains a subset of patients who fail TKI treatment and for whom the progression of CML from CP to AP and BC phases remains an all too relevant concern. Indeed, a recent follow up of the International Randomised Study of Interferon and ST1571 (IRIS) study has identified that almost 30% of patients ultimately discontinued IM due to suboptimal response or poor tolerance of therapy (Hochhaus *et al.*, 2009; Hughes *et al.*, 2010), and in single centre studies, this figure may be up to 50% (Gallipoli *et al.*, 2011). For some of these patients, second-generation TKI, such as nilotinib (NIL; Tasigna©, Novartis Pharmaceuticals) and dasatinib (DAS; Sprycel©, Bristol-Myers Squibb, Uxbridge, Middlesex, UK), may provide long-term disease control. However, these drugs also have significant potential toxicities, with 30–60% of patients experiencing grade 1–2 and 20% grade 3–4 toxicities in addition to considerable lifelong financial costs and the burden of living with a chronic disease (Druker *et al.*, 2006). Similarly, there remains the problems of secondary resistance and issues with drug compliance, with responses to second-generation TKI frequently incomplete with major molecular responses (MMR) of the order of 45% with NIL and DAS, and with any residual disease, there remains the persistent possibility of relapse (Giles *et al.*, 2010; Kantarjian *et al.*, 2010; Ibrahim *et al.*, 2011).

In addition, there is a significant population of patients who develop mutations within the kinase domain of BCR-ABL, including the well-documented T315I mutation. These mutations are due to different amino acid substitutions resulting in varying degrees of IM resistance, usually due to the inability of the TKI to bind to the kinase domain and thus affecting its activity (Gorre *et al.*, 2001; Branford *et al.*, 2002; Shah *et al.*, 2002). Third-generation TKI, ponatinib (PON; AP24534, Ariad Pharmaceuticals, Cambridge, MA, USA), is available, which is a potent inhibitor of BCR-ABL including in cells with the T315I mutation. The compound has shown acceptable safety, with evidence of anti-leukaemic activity with a major cytogenetic response (MCyR) found in 46% of patients in a phase I trial in patients with refractory CML (NCT00660920) (http://www.clinicaltrials.gov) (Cortes *et al.*, 2010; Zhou *et al.*, 2011). A phase II trial is currently underway (Ponatinib Ph + ALL and CML Evaluation, PACE, NCT01207440) in patients with CML resistant or intolerant to NIL or DAS, or with the T315I mutation (http://www.clinicaltrials.gov). The trial is still underway and full experimental data are required to show the activity of PON and any resulting adverse effects.

In addition to shortcomings with TKI as outlined earlier, there is overwhelming biological evidence that the leukaemic stem cell (LSC) fraction is not targeted by TKI, which acts to cause cell death in the proliferating mature cells only, therefore leaving the LSC pool able to repopulate the disease allowing disease persistence (Graham *et al.*, 2002). It is clear that additional therapy may therefore be required to target the LSC for disease eradication. It is known that BCR-ABL activation in the stem cell compartment results in the modulation of various signalling pathways. The TK activity of the oncoprotein leads to its autophosphorylation, ultimately leading to the modulation of various signalling pathways. This allows advantages to the LSC compartment in terms of cell survival, proliferation, differentiation and migration (Calabretta and Perrotti, 2004). In particular, BCR-ABL modulates key survival pathways, allowing the LSC to have a survival advantage and evade apoptosis in response to drug treatment. An alternative approach is therefore to exploit these survival pathways through the use of novel drugs to target members within the pathways. These drugs can then be used either alone or in combination with TKI to eradicate the disease. This review summarizes key pathways modulated by CML LSC and discusses current research on how components of these pathways can be targeted with novel drugs to manipulate LSC survival.

IFN

Prior to the implementation of TKI in CML treatment, IFN-α was found to induce cytogenetic responses in a proportion of CML patients and became first-line therapy for patients in CP (Kujawski and Talpaz, 2007). With drug combinations emerging as one potential treatment approach to provide an additive effect with TKI to target the LSC population, the use of IFN-α in combination with TKI has been hypothesized as an attractive therapy based on extensive literature demonstrating stem cell activity for IFN-α.

Various clinical trials have aimed to examine the additive effect of IFN-α in combination with TKI. The Preudhomme SPIRIT trial found a 'superior molecular response' defined as a four log reduction in BCR-ABL transcripts in the IM plus IFN-α group in comparison to IM alone (Preudhomme *et al.*, 2010) (NCT00219739) (http://www.clinicaltrials.gov). However, they also noted that a large percentage of patients in the IFN-α arm discontinued IFN-α due to side effects; therefore, dosage was halved and we await further experimental data with this lower dose.

Similarly, another group found that a large percentage of patients discontinued IFN-α due to drug-related toxicities (Simonsson *et al.*, 2011) (NCT00511121) (http://www.clinicaltrials.gov). However, in those in whom IFN-α was tolerated, a significantly higher percentage of MMR rates was seen in the IM plus IFN-α arm in comparison to single agent IM. This difference was marked in those who could tolerate the combination for at least 12 months.

In contrast to these studies, Cortes *et al*. (2011) found no benefit in terms of response to the combination of IM with IFN-α plus granulocyte-colony stimulating factor (G-CSF) (NCT00050531) (http://www.clinicaltrials.gov). However, no other studies have included G-CSF. In a series of over 1000 patients, Hehlmann *et al*. (2011) observed no improvement with the addition of IFN-α to IM. However, the trials discussed utilized different preparations of IFN-α with different dosing schedules, which may explain some of the disparities between the results. It is possible that a better effect will be seen in combination with alternative TKIs and clinical trials are underway in which CML patients in CP are tested for the effect of DAS or NIL in combination with IFN-α (NCT01657604; NCT01725204) (http://www.clinicaltrials.gov). However, it is clear the potential use of IFN-α as a stem cell therapy is somewhat limited by its incumbent toxicity.

In addition to the controversy in the literature on the benefit of IFN-α plus IM, the molecular mechanism of action against LSC is not well known. There is literature to suggest that IFN-α may elicit its effect due to activation of cell cycle in dormant stem cells, which has been shown on normal murine HSC *in vivo*. If this effect were paralleled in human LSC, IFN-α might make them more susceptible to the effects of TKI (Essers *et al*., 2009). However, any such effects in CML have not been documented. The use and efficacy of IFN in CML therapy is, however, clear and may be better explained by enhancement of the host immune system (Belardelli *et al*., 2002). Further information from clinical trials is needed, particularly regarding toxicity and to elucidate the mechanism of action which is likely to be complex.

Autophagy

Autophagy is a process involving the breakdown of intracellular components to aid survival, which occurs at a basal level at homeostasis or at enhanced levels in response to stress signals (Mizushima and Komatsu, 2011). In normal cells, low levels of basal autophagy are required for homeostasis. However, it is well documented that various types of cancer cells use autophagy as a defence mechanism to evade apoptosis (Kimmelman, 2011). In response to TKI treatment, CML LSC induces autophagy as a survival mechanism (Bellodi *et al*., 2009). Therefore, targeting the process of autophagy in combination with TKI treatment may provide an attractive therapy for eradicating LSC through blocking a fundamental survival pathway.

Drugs that are able to target components of the autophagy pathway include chloroquine (chloroquine diphosphate, CQ), which inhibits autophagy by induction of lysosomal stress (Maclean *et al*., 2008). Previous work has demonstrated that the combination of CQ with TKI treatment results in an additive reduction in colony numbers from cultured CD34 + CML cells (Bellodi *et al*., 2009). Similarly, a metabolite of CQ, hydroxychloroquine (HCQ), is available and shows similar additive effects in combination with TKI.

Currently, HCQ is being used in combination with cytotoxic chemotherapy in a number of clinical trials in both solid tumours and haematological malignancies (White and Dipaola, 2009). Based on positive experimental data, a phase II clinical trial is underway to investigate the potential benefits of HCQ in combination with IM in the treatment of CML in CP (chloroquine and imatinib combination to eliminate stem cells, CHOICES, Medical Research Council, NCT01227135) (http://www.clinicaltrials.gov). This trial is investigating the safety and efficacy of IM in combination with HCQ in CML patients in MCyR with residual disease. The biological data suggest that HCQ may provide an additive effect in promoting apoptosis in CML LSC and the clinical trial will assess whether efficacy can be demonstrated *in vivo* and whether tolerable HCQ doses are sufficient to inhibit the autophagy pathway and provide the necessary effect to eradicate the LSC. Indeed, a recent study noted an effective response to the combination of autophagy inhibitor clarithromycin and DAS *in vivo* in four patients with advanced CML with no issues with toxicity (Carella *et al*., 2012).

The autophagy pathway has clearly been identified as a key player in CML stem cell survival, and in addition to CQ and HCQ, investigators are examining the possibility of using alternative molecules to target autophagy. The autophagy pathway is complex involving interplay of several autophagy proteins and complexes. Possible drug targets include ULK1/2, VPS34, ATG4 and 7. Studies have already shown that targeting *ATG5* or *ATG7*, two essential autophagy genes, with RNA interference suppresses autophagy in CML cells resulting in a reduction in colony formation which is potentiated with IM (Bellodi *et al*., 2009). Future research is necessary to synthesize and test such compounds, and thus the use of autophagy inhibition in combination with TKI looks promising and exciting.

PI3K/AKT/mTOR pathway

A pathway that has been shown to interlink with autophagy signalling is the PI3K/AKT/mTOR pathway. This pathway is a major survival signalling pathway that plays roles in normal haemopoiesis and is mutated in both solid tumours and haematological malignancies, therefore representing an attractive therapeutic target (Wymann *et al*., 2003; Bader *et al*., 2005; Park *et al*., 2010). Importantly, the pathway has been shown to be deregulated in response to BCR-ABL activation and is modified in response to IM treatment (Skorski *et al*., 1995; Burchert *et al*., 2005).

Rapamycin (Rapamune©, Pfizer, New York, NY, USA) is an efficient inhibitor of pathway component mTOR and is emerging as a potentially interesting anti-cancer compound. It functions as an allosteric inhibitor of the mTORC1 component. K562 cells treated with rapamycin showed a decrease in viability and an increase of cells in G0/G1 phases of cell cycle through modulation of phosphorylation of mTOR (Li *et al*., 2012b). A small-scale study treated six patients with IM-resistant CML with rapamycin and showed minimal toxicity with a transient decrease in BCR-ABL transcripts; however, no MMR or MCyR were achieved (Sillaber *et al*., 2008). A larger study conducted over a longer time period would be required to fully examine whether rapamycin has potential as an appropriate treatment for the treatment of CML.

In addition to rapamycin, there are several compounds available that target different components of the PI3K/AKT/

mTOR pathway. GDC0941 (Chemdea, Ridgewood, NJ, USA), a pan PI3K inhibitor, and KU-0063794 (Chemdea), a dual mTORC1/2 inhibitor, have been shown to suppress colony formation in murine cells derived from mice that have undergone reconstitution with HSC transduced with BCR-ABL (Schuster *et al*., 2011).

Alternatively, compounds are also available, which target combined components of the pathway. A particularly successful compound is NVP-BEZ235 (Novartis Pharmaceuticals), which functions as a dual PI3K and mTORC1/2 inhibitor. This compound suppresses colony formation of murine cells derived from mice that have undergone reconstitution with HSC transduced with BCR-ABL (Schuster *et al*., 2011). Furthermore, the compound shows an antiproliferative effect on BCR-ABL expressing BA/F3 cells and IM-resistant mutants, while showing little activity against parental cells. The same group also demonstrated an antileukaemic effect *in vivo* in a CML mouse model. However, there is controversy as to whether the effects of dual PI3K and mTOR inhibitors are greater as compared to inhibition of mTOR alone (Wong *et al*., 2011). Clinical trials are currently underway using NVP-BEZ235 in other cancer settings and further experimental data are required to further elucidate the advantage of this compound, and others in its class in CML therapy (Maira *et al*., 2010).

FOXO and TGF-β

BCR-ABL is known to activate the PI3K/AKT/mTOR pathway, which, in turn, suppresses the expression of Forkhead O (FOXO) transcription factors (Jagani *et al*., 2008). This results in pro-proliferative and anti-apoptotic effects in CML cells. Recent data suggest that targeting the FOXO signalling pathway, in particular through TGF-β inhibition, might represent an effective therapy in CML.

A compelling study by Naka *et al*. (2010) showed that when CML was induced on a FOXO3 null haemopoietic background, LSC functions were compromised. The effect of BCR-ABL on FOXO was shown to be modulated via TGF-β signalling. The authors used a CML-like myeloproliferative mouse model to show that the combination of TGF-β inhibition using Ly364947 (Sigma-Aldrich, Gillingham, Dorset, UK), FOXO3 deficiency and IM treatment, leads to CML depletion *in vivo*. Further independent research identified BCL6 as a critical effector downstream of FOXO (Hurtz *et al*., 2011). BCL6 up-regulation by TKI treatment repressed Arf and p53 in CML cells and peptide inhibition of BCL6 in human CML cells reduced colony formation and initiation of leukaemia in transplantation assays. Importantly, inhibition of BCL6 was able to selectively eradicate the most primitive, quiescent LSC fraction.

TGF-β is a popular pharmacological target. TGF-β targeting using small-molecule inhibitors, peptides or antibodies has become a popular approach in a variety of alternative disease settings, many of which have reached phase III clinical trials (Akhurst and Hata, 2012). In the context of CML, a study by Moller *et al*. (2007) used TGF-β inhibition using SB431542 (Tocris Bioscience, Bristol, UK) to inhibit the growth of CD34 + CML CP samples and to enhance cell death in combination with IM in non-proliferating CML cells.

The use of anti-BCL6 peptides and TGF-β inhibition could provide a novel therapy for targeting the LSC population. Furthermore, FOXO inhibitors have recently become available and future research will assess whether they are effective in CML therapy.

Acetylation

Epigenetic therapies are emerging as an exciting novel approach with enormous potential in the fight against cancer. There are numerous epigenetic modifications of nucleosomes, but the two currently receiving the most attention in terms of drug development are the application of histone deacetylase (HDAC) inhibitors (HDACI) and hypomethylating agents. In particular, HDACI have been under investigation for relevant activity in CML. Research on HDACI in CML is growing and shows great potential, in particular as there is evidence HDACI can target non-proliferating tumour cells (Burgess *et al*., 2004).

Epigenetic alterations participate in the development of acquired resistance to TKI treatment, such as with IM. IM-resistant K562 cells have been shown to display aberrant acetylation of non-histone proteins due to up-regulation of HDAC and down-regulation of histone acetyltransferase (Lee *et al*., 2007). The authors then showed that IM-resistant K562 cells were more sensitive to the HDACI suberoylanilide hydroxamic acid (SAHA; Vorinostat, Merck Pharmaceuticals, Whitehouse Station, NJ, USA) than parental K562 cells. SAHA has been shown to have activity against numerous tumour types, either as a single agent but perhaps more impressively as part of combination therapy. SAHA down-regulates BCR-ABL levels and causes a dose dependent increase in apoptosis of K562 cells which is potentiated with the addition of IM (Yu *et al*., 2003; Nimmanapalli *et al*., 2003b). Furthermore, SAHA has been shown to increase the activity of the BCR-ABL/aurora kinase inhibitor MK-0457 (Tozasertib, Merck Pharmaceuticals), against both IM sensitive and resistant CML cells (Dai *et al*., 2008). SAHA was approved by the Food and Drug Administration (FDA) in 2006 for the treatment of advanced cutaneous T-cell lymphoma (Duvic *et al*., 2007; Mann *et al*., 2007; Olsen *et al*., 2007) and has been shown in phase I clinical trials to be well tolerated in patients (Garcia-Manero *et al*., 2008).

Several additional HDACI are currently available and show evidence of targeting CML cells. Treatment with LAQ824 (Novartis Pharmaceuticals) was effective with the combination with IM in inducing apoptosis in CML BC CD34+ cells (Nimmanapalli *et al*., 2003a). Interestingly, LAQ824 down-regulated BCR-ABL levels in CML BC cells possessing the T315I mutation and induced apoptosis in IM refractory primary CML BC cells.

NIL and HDACI LBH589 (Panobinostat, Novartis Pharmaceuticals) induced apoptosis in IM-resistant cell lines expressing kinase domain mutants, through reduction in BCR-ABL levels (Fiskus *et al*., 2006). Treatment with LBH589 also depleted BCR-ABL levels and induced apoptosis of IM resistant primary human CML cells, including those expressing the T315I mutation. This effect was potentiated with the addition of NIL. LBH589 is currently in a phase I clinical trial in combination with IM investigating the safety and maximal

tolerated dose for patients with previously treated CML in CP (NCT00686218, City of Hope Medical Centre) (http://www.clinicaltrials.gov).

A recent study found that HDACI valproic acid exhibited strong anti-proliferative and pro-apoptotic effects on CML cell lines at concentrations achievable *in vivo* (Kircher *et al.*, 2009). The compound also inhibited the growth of colony-forming cells from CML patients, but showed activity against normal progenitors indicating a lack of selectivity. It should be borne in mind that the anti-cancer effects of first generation HDACI are not simply restricted to increased acetylation of histones and subsequent changes in chromatin structure. HDAC also deacetylate transcription factors and other regulatory proteins and some HDACI have broader effects beyond inhibition of deacetylation. Lack of specificity of these agents is therefore a cause for concern.

These agents, however, represent early drug development efforts in this field and already more targeted epigenetic therapies are emerging. BET bromodomain inhibitors target chromatin 'readers' with several small molecules now in development that specifically target protein–protein interactions of specific epigenetic readers. These include an inhibitor of BRD2, which has been shown to down-regulate c-Myc and its target genes. This is clearly a highly desirable therapeutic strategy across many tumour types, although, to date, the most significant pre-clinical activity appears to be in haemopoietic malignancies (Dawson *et al.*, 2012). This rapidly expanding field of anti-cancer therapeutics holds a tangible potential now for provision of novel stem cell therapies in CML.

CXCR4/CXCL12 axis

Chemokine receptor CXCR4 and ligand CXCL12 (SDF-1) represent a binding partnership that is well known in haemopoiesis. Studies using CXCR4 and CXCL12 null mice demonstrated the indispensible role of these proteins at an early stage of haemopoiesis (Nagasawa *et al.*, 1996; Tachibana *et al.*, 1998; Zou *et al.*, 1998; Bagri *et al.*, 2002; Lieberam *et al.*, 2005).

The CXCR4/CXCL12 axis is thought to be important for normal HSC migration to and from the bone marrow niche. BCR-ABL activity results in down-regulation of CXCR4 expression and defective adhesion of CML cells to bone marrow stroma (Jin *et al.*, 2008). TKI treatment with IM on K562 and CML progenitor cells was found to increase CXCR4 expression and lead to migration of CML cells to the bone marrow microenvironment niche, where CML cells may reside in a protected state. CXCR4 inhibitors might therefore overcome this LSC protection by the bone marrow microenvironment, rendering them vulnerable to eradication by TKI.

CXCR4 is a G-protein coupled receptor which as a class has been popular in drug development due to the relative ease of production of small-molecule inhibitors. Small-molecule inhibitor of CXCR4, AMD3100 (Plerixafor, Mozobil®, Genzyme Therapeutics Ltd, Oxford, UK), acts as an efficient stem cell mobilization agent through blocking CXCR4 in an active confirmation so ligand CXCL12 cannot bind (Uy *et al.*, 2008).

Due to the modulation of CXCR4 in BCR-ABL cells and its normalization in response to TKI treatment, researchers have aimed to target CXCR4 signalling in CML using AMD3100. AMD3100 was found to diminish BCR-ABL+ cell migration and reduce adhesion of these cells to extra cellular-matrix components and to BM stromal cells *in vitro* (Weisberg *et al.*, 2012). AMD3100 in combination with NIL was found to reduce leukaemia in mouse models below the baseline of NIL alone. These data suggest that CXCR4 inhibition in combination with TKI can override some aspects of drug resistance in CML and may aid suppression/eradication of residual disease. AMD3100 is currently in a phase I clinical trial by Genzyme in CML patients to determine the maximum tolerated dose of AMD3100 (NCT01068301) (http://www.clinicaltrials.gov). Future work will assess the tolerance of AMD3100 and whether there is an added benefit in combination with TKI treatment *in vivo*.

Wnt/β-catenin pathway

The Wnt/β-catenin signalling pathway is known to play essential roles in a variety of different processes in haemopoiesis, including both proliferation and self-renewal, and its disruption is associated with development of haematological malignancies (Seke Etet *et al.*, 2012). BCR-ABL is known to directly activate this signalling pathway, which has been shown to be important during various stages of CML. Targeting components of this pathway should therefore target key properties of the LSC. Potential candidates include Wnt/β-catenin inhibitors aiming to inhibit self-renewal and also glycogen synthase kinase 3 (GSK3) inhibitors that would activate the pathway.

β-Catenin is a key effector molecule in the canonical Wnt pathway and in haemopoiesis. Mice that lack β-catenin in their haemopoietic cells provided evidence for the critical role of this signalling pathway in haemopoiesis and in CML (Zhao *et al.*, 2007). Mice deficient in β-catenin could form HSC but the cells could not be maintained long-term. A study by Jamieson *et al.* (2004) showed that the granulocyte-macrophage progenitor pool from patients with CML BC and IM resistant CML had elevated levels of nuclear β-catenin as compared with normal marrow. It has been shown that BCR-ABL physically interacts with β-catenin and controls levels of protein stabilization in CML cells (Coluccia *et al.*, 2007). Furthermore, β-catenin has been shown to play a crucial role in the survival of LSC, which are resistant to IM (Hu *et al.*, 2009).

A key paper by Heidel *et al.* (2012) reports the genetic deletion of β-catenin in a CML mouse model. Interestingly, deletion of β-catenin after the initiation of CML does not increase survival. However, deletion of β-catenin shows a synergistic effect with IM to delay disease recurrence after IM discontinuation and to abrogate CML stem cells. Recently, it has been shown that targeting prostaglandin signalling via COX inhibitors can abrogate β-catenin levels, both in HSC, AML and CML LSC (Goessling *et al.*, 2009; Wang *et al.*, 2010; Heidel *et al.*, 2012). Pharmacological inhibition of β-catenin via modulation of prostaglandin signalling using indomethacin leads to a reduction in LSC (Heidel *et al.*, 2012).

A novel Wnt/β-catenin inhibitor AV65 was shown to inhibit proliferation of CML cell lines, including cells with

the T315I mutation present (Nagao *et al.*, 2011). This inhibitor was shown to reduce the expression of β-catenin in CML cells and to induce apoptosis. The combination with IM showed a synergistic effect on CML cell proliferation.

SB216763 (Sigma-Aldrich) is an ATP competitive inhibitor of GSK3 and has been shown to induce apoptosis in CML stem/progenitor cells with the addition of IM (Reddiconto *et al.*, 2012). Furthermore, SB216763 was shown to reduce CML long-term culture initiating cells (LT-CIC) without affecting normal cells. This effect was potentiated with the addition of IM. These compounds are suggested to act to induce cycling in CP CML cells, which are then targeted with TKI treatment.

The use of Wnt/β-catenin/GSK3 inhibitors is therefore of interest, appearing to show promising results in CML. However, the precise functional role of this pathway in both normal and malignant haemopoiesis remains somewhat controversial. Recent reports suggest that Wnt signalling regulates normal haemopoiesis in a dosage-dependent fashion (Luis *et al.*, 2011). Furthermore, data show that the pathway is important not only for HSC, but for other cells within the niche. Therefore, due to complicated niche interactions, further experimental data are required to fully understand the mechanisms involved and to demonstrate that targeting this pathway will not be detrimental to normal cells.

JAK-STAT signalling

The JAK-STAT signalling pathway is responsible for a variety of different functions throughout development. There is an abundance of literature to suggest that this pathway is activated in CML LSC and family member JAK2 is a popular pharmacological target.

JAK are a family of intracellular, non-receptor TK that transduce cytokine-mediated signals via the JAK/STAT pathway. JAK2 has been implicated in many biological functions, including haemopoiesis. Studies on JAK2 null mice identified JAK2 as a fundamental player in normal haemopoiesis (Neubauer *et al.*, 1998; Parganas *et al.*, 1998). Several studies then identified JAK2 as a key player in a variety of myeloproliferative disorders through the identification of JAK2 mutations present in patients (Levine *et al.*, 2007).

JAK2 has recently emerged also as a hot topic in CML research. JAK2 is phosphorylated in cytokine independent BCR-ABL+ cells (Xie *et al.*, 2002) (Xie *et al.*, 2001). Knockdown of JAK2 in IM-resistant K562 cell lines resulted in reduced colony formation; therefore, JAK2 inhibitors may be applicable for CML therapy (Samanta *et al.*, 2006). It has been suggested that the mechanism of action of JAK2 in CML is via Lyn kinase through the SET-PP2A-SHP1 pathway (Samanta *et al.*, 2009).

AG490 (LC Laboratories, Woburn, MA, USA) is a potent JAK2 inhibitor. Treatment of CML IM sensitive and resistant cell lines and mouse BCR-ABL+ 32D cells resulted in the reduction of c-MYC and inhibited cell survival (Samanta *et al.*, 2006). AG490 induced apoptosis in bone marrow cells derived from CML patients in all phases of disease. AG490 was shown to be effective against cells from patients who failed IM and did not significantly affect progenitors from healthy donors (Samanta *et al.*, 2009).

JAK2 inhibitors TG101209 (TargeGen, Inc., San Diego, CA, USA) and HBC (1,2,3,4,5,6-hexabromocyclohexane; EMD Chemicals, Darmstadt, Germany) were shown to induce apoptosis in IM sensitive and resistant cell lines and a combination of HBC and IM significantly induced apoptosis in BC CML primary cells (Samanta *et al.*, 2009). A study by Traer *et al.* (2012) used a co-culture system in which CML cell lines and primary cells were cultured in the presence of medium derived from a stromal layer which secretes soluble factors designed to mimic the *in vivo* microenvironment. This study examined the efficacy of JAK2 inhibitors in the presence of conditioned medium and found that TG101209 and JAK1/2 inhibitor CYT387 (Stratech Scientific Ltd., Suffolk, UK), in combination with IM, reduced the anti-apoptotic effect found with conditioned medium alone. JAK2 inhibitor TG101209 was then tested *in vivo* in a CML mouse model. Mice treated with the JAK2 inhibitor alone showed a modestly prolonged survival in comparison to vehicle alone. The combination effect with NIL was more effective against BCR-ABL+ cells, however toxicity using a higher dose of TG101209 was noted, while the lower dose of TG101209 with the combination showed no advantage over NIL alone.

A new dual kinase inhibitor for JAK2 and ABL kinases, ON044580 (synthesized by Dr. Reddy) (Jatiani *et al.*, 2010), targets BCR-ABL+ IM sensitive and resistant K562 cells and cells from BC CML patients (Samanta *et al.*, 2010).

Recent research aimed to elucidate the role of JAK2 in BCR-ABL driven leukaemia. To achieve this JAK2 was deleted in the HSC system and BCR-ABL dependent transformation was examined (Hantschel *et al.*, 2012). Interestingly, the authors noted that myeloid transformation and leukaemia maintenance were independent of JAK2. Furthermore, JAK2 inhibitors were shown to induce apoptosis irrespective of JAK2 expression in cell lines with conditional JAK2 knockout, therefore questioning the specificity of these inhibitors. The combination of JAK2 inhibitors with TKI have been shown to be effective against CML cell lines and primary cells, however further work is still required to assess the effectiveness, toxicity and specificity of inhibitors.

Hedgehog pathway

The Hedgehog (Hh) pathway was discovered and named due to the role of the pathway in *Drosophila melanogaster* development (Nusslein-Volhard and Wieschaus, 1980). The pathway plays a variety of different roles in various cell types and is disrupted in several cancers (Raju and Pham, 2012). Studies have indicated that components of the Hh pathway are modulated in CML LSC in comparison to normal counterparts.

The Hh pathway is complex with signalling ligands, transmembrane receptors and various intracellular proteins (Raju and Pham, 2012). Simply, Hh ligands bind receptor Patched, which relieves repression on transmembrane protein smoothened (SMO), leading to a signal transduction cascade resulting in nuclear translocation of Gli transcription factors and various downstream effects. An ideal pharmacological target is transmembrane protein SMO which transduces the signalling cascade. There is growing evidence that targeting SMO in CML may indeed be an appropriate target.

SMO is activated in BCR-ABL+ LSC in comparison to normal counterparts (Dierks *et al*., 2008). SMO null mice crossed with a CML mouse model show a reduction in disease incidence (Zhao *et al*., 2009). In contrast, an overexpression of SMO with a CML mouse model results in an increase of LSC, which leads to a significant increase in disease progression. However, the Hh pathway plays an important role in normal haemopoiesis and conditional deletion of SMO in the normal haemopoietic system reduces long-term HSC function in primary and secondary transplants (Zhao *et al*., 2009).

SMO inhibitor cyclopamine (Toronto Research Chemicals, Toronto, Canada) is a plant-derived steroidal alkaloid that binds to SMO, stabilizing the protein in an inactive form, therefore inhibiting Hh signalling (Taipale *et al*., 2000). Exposure to cyclopamine was found to inhibit leukaemic colony growth *in vitro* and to prolong survival in a CML mouse model (Dierks *et al*., 2008; Zhao *et al*., 2009). In addition, treatment was reported to reduce colony growth in IM resistant cell lines and BC CML primary cells.

Several SMO inhibitors are now available including LDE225 (Novartis Pharmaceuticals). LDE225 in combination with NIL reduced CP CML colony formation in secondary replates (Irvine *et al*., 2009). Furthermore, LDE225 in combination with NIL showed promising results in a transgenic BCR-ABL mouse model of CP CML (Zhang *et al*., 2010). A phase I clinical trial is currently underway led by Novartis Pharmaceuticals investigating the feasibility of administrating the combination of LDE225 with NIL to patients with CML who have failed other TKI treatments (NCT01456676) (http://www.clinicaltrials.gov). The use of SMO inhibitors in CML looks promising; however, further research should focus on normal counterparts due to the extensive roles of the pathway, in particular in normal HSC biology.

ALOX5

Arachidonate 5-lipoxygenase (5-LO) (ALOX5) has been shown to be important in several processes including oxidative stress response and, more recently, cancer. ALOX5 is regulated by BCR-ABL and is suggested to play a role in disease initiation and progression with pharmaceutical inhibition showing promising data in CML.

Chen *et al*. (2009) aimed to elucidate the role of ALOX5 in CML. ALOX5 was shown to be up-regulated by BCR-ABL, in terms of both gene expression and function, and this up-regulation was not abolished by IM, suggesting kinase independence. CML was not initiated on an ALOX5 null background, suggesting a fundamental role in disease initiation. This was confirmed using co-expression of BCR-ABL with ALOX5 which rescued the defective CML phenotype seen in the ALOX5null mice. Importantly, the authors showed that ALOX5 deficiency impaired LSC function without affecting normal HSC. Recent data suggested that ALOX5 functions through Msr1, which itself has now been shown to affect LSC function and CML development (Chen *et al*., 2011).

Importantly for therapy, ALOX5 is targetable with inhibitors. Zileuton is an ALOX5 inhibitor (Zyflo, Cornerstone Therapeutics Inc., Cary, NC, USA). Zileuton inhibited ALOX5 function in CML mice and was more effective when used alone in comparison to IM treatment alone (Chen *et al*., 2009). Interestingly, CML mice treated with both Zileuton and IM showed an additive effect with prolonged survival. Based on these positive data, Zileuton is currently in a phase I study to evaluate the safety of the combination of Zileuton in combination with IM in CML patients (NCT01130688) (http://www.clinicaltrials.gov).

Future research will focus on the development of more potent and specific ALOX5 inhibitors and whether alternative arachidonate lipoxygenases may also be targeted for benefit in CML.

Promyelocytic leukaemia protein

The promyelocytic leukaemia protein (PML) has been shown to have a critical role in haemopoiesis, which is deregulated in CML and this protein can be targeted with arsenic compounds.

PML is a protein that controls fundamental processes such as apoptosis, cellular proliferation and senescence (Salomoni *et al*., 2012). PML is highly expressed in the HSC compartment and is expressed at higher levels in samples from CP CML patients (Ito *et al*., 2008). Interestingly, high PML expression correlated with lower CMR and CCyR as compared to patients with low levels of PML, suggesting an important link to prognosis. In other settings, including the haemopoietic system, the PML protein acts as a tumour suppressor (Rego *et al*., 2001; Virador *et al*., 2009). However, in this case, high levels are associated with leukaemia, suggesting PML in the context of CML is not functioning as a tumour suppressor and requires further experimental data. Ito *et al*. (2008) showed that the protein PML was indispensable for LSC maintenance. However, studies using PML null mice also showed that the protein affects normal haemopoiesis with PML null mice showing a lack of long-term repopulating capacity.

Arsenic trioxide (As_2O_3) is a well-known compound used in leukaemia therapy. As_2O_3 degrades PML protein, making it an ideal compound for CML therapy as PML is expressed at high levels and high levels are associated with poor prognosis. As_2O_3 significantly decreased the number of quiescent LSC and the compound had an inhibitory effect on LSC in long-term culture assays (Ito *et al*., 2008). Administration of As_2O_3 in combination with Ara-C in mice transplanted with cells transduced with BCR-ABL resulted in apoptosis in the LSC compartment. The authors suggested this was due to the reduction of HSC quiescence by As_2O_3. However, it should be noted that the compound was found to decrease PML expression in normal mouse HSC, which resulted in the reduction in colony forming capability *in vitro* and impaired HSC quiescence and stem/progenitor frequency (Ito *et al*., 2008). However, the authors suggested that As_2O_3 induced apoptosis at higher levels in CML cells than normal HSC. Treatment of primary CML cells showed an induction in apoptosis with As_2O_3 which was potentiated with the addition of Ara-C. Recent research indicated that the anti-leukaemic effects of As_2O_3 may involve autophagic degradation of BCR-ABL (Goussetis *et al*., 2012). A phase II study to test the activity of As_2O_3 in combination with IM in CML patients with evidence of residual disease has been completed by the New

Mexico Cancer Care Alliance (http://www.clinicaltrials.gov) (NCT00250042) with results eagerly anticipated. A further phase I trial, led by the Beth Israel Deaconess Medical Centre, is examining the combination of As_2O_3 with IM, DAS or NIL (http://www.clinicaltrials.gov) (NCT01397734). The compound seems to be effective; however, the noted toxicity and effect on cell cycle found on normal HSC will need to be monitored.

Alternatively, arsenic compound arsenic sulphide (As_4S_4) in combination with IM showed a therapeutic effect in a BCR-ABL+ mouse model of CML (Zhang *et al.*, 2009). A systems biology approach was undertaken to elucidate the mechanism of action for the combination and found that As_4S targeted BCR-ABL through ubiquitination of key lysine residues leading to proteasomal degradation, while IM predominantly inhibited the PI3K/AKT/mTOR pathway. The combination of both compounds led to cell cycle arrest, decreased activity of BCR-ABL and activated apoptosis pathways in CML stem cells.

Protein phosphatase 2A

Data suggest that re-activation of tumour suppressor protein protein phosphatase 2A (PP2A) may be a useful approach in CML therapy. PP2A is down-regulated in CML LSC in comparison to normal HSC and a pharmacological agent which activates PP2A has been shown to eradicate CML LSC.

Perrotti's group has led encouraging studies demonstrating the relevance of PP2A in BCR-ABL driven leukaemia and shown that a pharmacological activator may provide an effective therapy in CML patients. PP2A is reduced in CD34 + CML progenitors in comparison to normal cells (Neviani *et al.*, 2005). It has been shown that PP2A is inhibited in the primitive CD34 + CD38- cell fraction from CML patients (Oaks *et al.*, 2009). The inactivation of PP2A in CML cells is due to the enhancement of PP2A inhibitor protein SET in response to BCR-ABL activity. BCR-ABL induces the expression of inhibitor protein SET in a dose and kinase-dependent manner and recent evidence suggests that JAK2 has a role in activating SET downstream of BCR-ABL (Samanta *et al.*, 2009). CIP2A is an inhibitor of PP2A and higher levels of CIP2A correlate with progression to BC; therefore, the PP2A protein is clinically important for prognosis (Lucas *et al.*, 2011).

Pharmacologically targeting PP2A for activation may provide an effective treatment in CML. FTY720 (Fingolimod, Gilenia®, Novartis Pharmaceuticals) is a potent PP2A activator that has recently received FDA approval for relapsing multiple sclerosis (Cohen *et al.*, 2010) where its benefits are thought to be gained through its effects on lymphocyte trafficking. FTY720 is currently undergoing pre-clinical investigation in solid tumours and haematological malignancies (Alinari *et al.*, 2012; Estrada-Bernal *et al.*, 2012; Mousseau *et al.*, 2012; Yang *et al.*, 2012; Li *et al.*, 2012a).

FTY720 has been shown to induce apoptosis and impair clonogenicity of TKI sensitive and resistant myeloid cell lines and CML BC primary cells, while sparing normal cells (Neviani *et al.*, 2007). FTY720 was shown to decrease the number of leukaemic LTC-IC and more importantly quiescent LSC (Oaks *et al.*, 2009). Treatment with FTY720 *in vivo* reduced the leukaemic burden in a CML mouse model, the number of long-term repopulating HSC and impaired engraftment in secondary recipients in comparison to untreated leukaemic mice (Neviani *et al.*, 2010).

Although the anti-leukaemic properties of FTY720 have been documented to be secondary to reactivation of PP2A, this compound also induces caspase-independent leukaemic cell death, suggesting other mechanisms are also relevant. FTY720 is also an inhibitor of sphingosine 1 phosphate (SIP), a bioactive lipid that is a key player in many biological processes including cell survival, proliferation and migration (Pyne *et al.*, 2011). Moreover, it has been reported that elevated levels of SIP can lead to IM resistance in CML (Baran *et al.*, 2007) and that inhibition of sphingosine kinase 1 (SK1, an enzyme that catalyses formation of SIP from sphingosine) can overcome this resistance through inhibition of the RAS/MAPK signalling pathway in BC cell lines (Bonhoure *et al.*, 2008). FTY720 is therefore an attractive potential novel agent against CML stem cells, potentially curbing several mechanisms of carcinogenesis simultaneously.

A recent study used a novel, specific cell penetrating peptide OP449 that binds to SET and antagonises inhibition of PP2A by SET (Agarwal *et al.*, 2011). OP449 showed an increase in PP2A activity in human and mouse BCR-ABL+ cell lines, with a greater potency than FTY720. Furthermore, OP449 reduced proliferation and potently increased apoptosis in cell lines, with a greater potency against BCR-ABL+ cell lines in comparison to parental cells. Experiments on primary CD34 + CML cells showed a reduction in colony formation with OP449 treatment with no toxicity in normal cells, which was potentiated with the addition of TKI. The SK1/SIP/PP2A pathway has therefore been shown to be relevant in CML and FTY720 and analogous agents look promising in terms of pre-clinical data.

Cyclopentenone prostaglandins

There is emerging evidence that long-chain polyunsaturated fatty acids (PUFA) found in products of marine origin have anti-cancer properties (Hajjaji and Bougnoux, 2012). The rationale for these agents came from strong epidemiological evidence linking fish oil with low incidences of several tumour types (Kaizer *et al.*, 1989; Berg *et al.*, 1994; Schloss *et al.*, 1997). There is also an increasing epidemiological premise for these natural metabolites in cardiovascular disease prevention (Szymanski *et al.*, 2010).

Naturally produced cyclopentenone prostaglandins (CyPG) are available, which are produced from the dietary fish-oil ω-3 PUFA eicosapentaenoic acid (EPA). Hegde *et al.* (2011) have recently shown that metabolites of EPA 15d-PGJ_2 and Δ^{12}-PGJ_3 exhibit activity against LSCs (Hegde *et al.*, 2011).

The pro-apoptotic activity of 15d-PGJ_2 activity was previously reported against AML and CML stem cells by Hassane *et al.* who employed an *in silico* study using cDNA microarray expression profiles available from the Gene Expression Omnibus database, identifying 15d-PGJ_2 as a compound that abrogates LSCs (Hassane *et al.*, 2008). Shin *et al.* (2009) showed that 15d-PGJ_2 exerts its anti-leukaemic effects in the HL-60 cell line through increased production of reactive

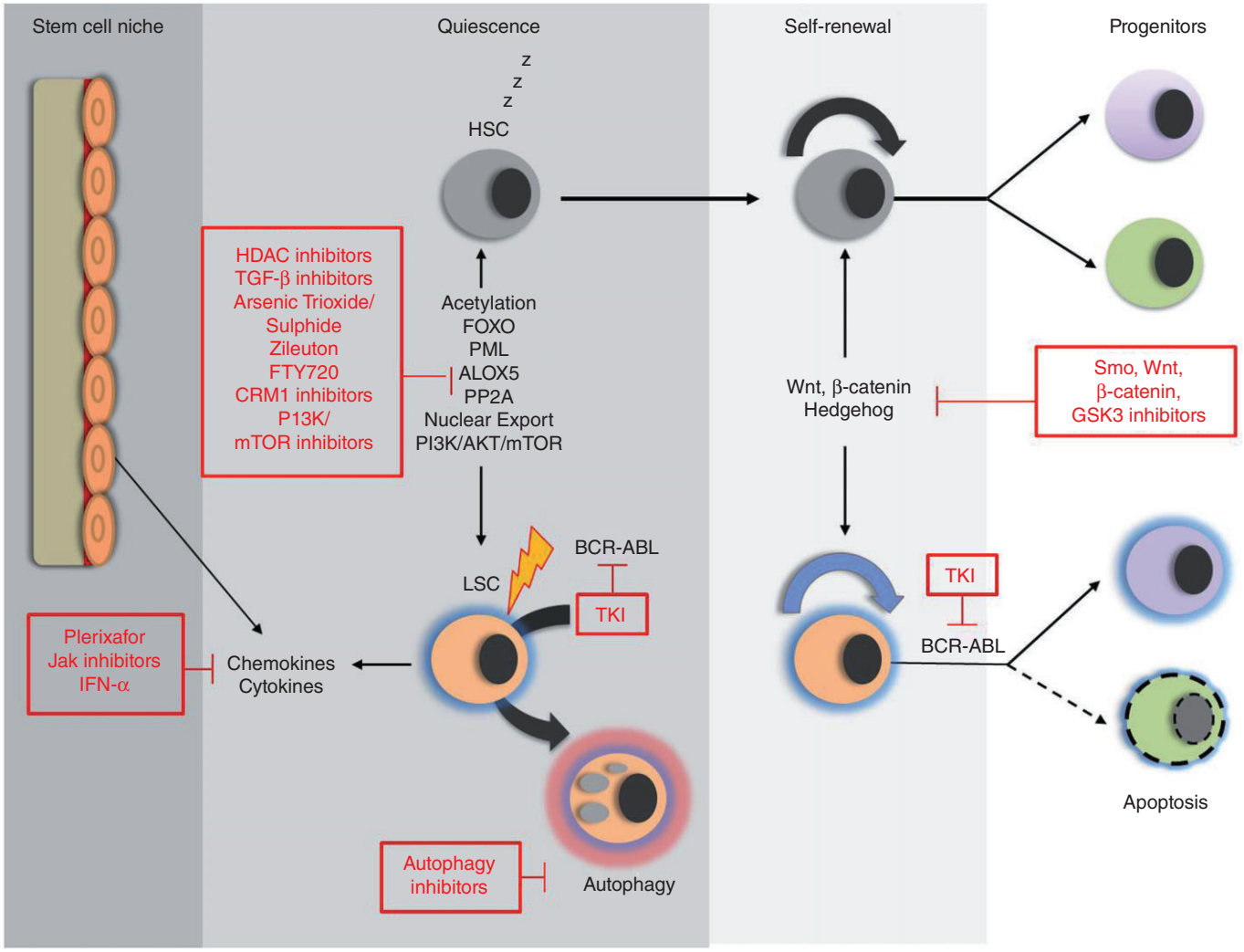

Figure 1

Schematic overview of signalling pathways and drug targets currently under investigation in CML research. Standard therapy against BCR-ABL with TKI effectively inhibits the kinase activity of the oncoprotein and results in apoptosis in the more mature progenitor cells; however, the quiescent LSCs are less sensitive to treatment and studies have shown these cells undergo survival pathway autophagy. Based on positive pre-clinical data, autophagy inhibitors are being investigated in combination with TKI treatment. The transformation of a HSC into a LSC alters key processes including acetylation, nuclear transport and the expression of genes including FOXO, PML, ALOX, PP2A and PI3K/Akt/mTOR pathway. Several inhibitors/activators of these genes/pathways are under investigation alone and in combination with TKI treatment. Normal HSC undergo self-renewal and the key self-renewal genes have been identified as playing a role in CML. Targets of the key pathways including Wnt, β-catenin and hedgehog are currently under investigation. Chemokine/cytokine signalling involving the HSC and accessory cells within the bone marrow stem cell niche is altered in CML and compounds are being tested to target this signalling using inhibitors against CXCR4 and JAK. IFN-α in combination with TKI shows promising data in CML; however, the mechanism of action is not well understood and is likely to be complex, involving other cell types and niche interactions.

oxygen species. Hegde *et al.*, however, used significantly lower concentrations of CyPGs and did not find any increase in ROS, but reported that activation of the ATM-p53 apoptosis pathway was the mode of eradication of the LSCs.

A mouse model of CML shows that Δ^{12}-PGJ_3 administration eliminated leukaemia and restored normal haemopoiesis in a BCR-ABL+ mouse model of CML. In particular, the drug selectively targeted the LSC fraction for apoptosis (Hegde *et al.*, 2011). In addition, secondary transplants from treated mice failed to develop leukaemia corroborating that the LSC had been eradicated by the CyPG. Treatment of CML mice with IM then Δ^{12}-PGJ_3 enhanced survival and prevented relapse. Interestingly, despite the marked pro-apoptotic effects seen on LSC, reassuringly, no adverse effects on HSC or downstream progenitors were reported.

The recent work by Hegde *et al.* provides a compelling study suggesting that the current increasing excitement regarding these natural compounds, across several branches of medicine, is very relevant to leukaemia research and seems to be a promising therapy for CML.

Table 1

Table of signalling pathways and compounds currently under investigation in CML research

Signalling pathway	Compound	Mechanism of action	Stage	Clinical trial identifier	References
IFN	IFN-α	Unknown	Phases II and III	NCT00219739; NCT00511121; NCT01657604; NCT00050531; NCT01725204	Preudhomme *et al.*, 2010; Cortes *et al.*, 2011; Hehlmann *et al.*, 2011; Simonsson *et al.*, 2011
Autophagy	Chloroquine	Antophagy inhibitor	Pre-clinical		Bellodi *et al.*, 2009
	Hydroxychloroquine	Autophagy inhibitor	Phase II	NCT01227135	Bellodi *et al.*, 2009
	Clarithromycin	Autophagy inhibitor	Pre-clinical		Carella *et al.*, 2012
P13K/AKT/mTOR	Rapamycin	mTORC1 inhibitor	Pre-clinical		Li *et al.*, 2012b, Sillaber *et al.*, 2008
	GDC0941	P13K inhibitor	Pre-clinical		Schuster *et al.*, 2011
	KU-0063794	Dual mTORC1/2 inhibitor	Pre-clinical		Schuster *et al.*, 2011
	NVP-BEZ235	Dual P13K and mTORC1/2 inhibitor	Pre-clinical		Schuster *et al.*, 2011
FOXO/TGF-β	Ly364947	TGF-β inhibitor	Pre-clinical		Naka *et al.*, 2010
	SB431542	TGF-β inhibitor	Pre-clinical		Moller *et al.*, 2007
Acetylation	SAHA	Histone deacetylase inhibitor	Pre-clinical		Nimmanapalli *et al.*, 2003b; Yu *et al.*, 2003; Lee *et al.*, 2007; Dai *et al.*, 2008
	LAQ824	Histone deacetylase inhibitor	Pre-clinical		Nimmanapalli *et al.*, 2003a
	LBH589	Histone deacetylase inhibitor	Phase I	NCT00686218	Fiskus *et al.*, 2006
	Valproic acid	Histone deacetylase inhibitor	Pre-clinical		Kircher *et al.*, 2009
CXCR4/CXCL12	Plerixafor	CXCR4 inhibitor	Phase I	NCT01068301	Jin *et al.*, 2008; Weisberg *et al.*, 2012
Wnt/β-catenin	Indomethacin	β-catenin inhibitor	Pre-clinical		Heidel *et al.*, 2012
	Av65	Wnt/β-catenin inhibitor	Pre-clinical		Nagao *et al.*, 2011
	SB216763	GSK3 inhibitor	Pre-clinical		Reddiconto *et al.*, 2012
JAK-STAT	AG490	JAK2 inhibitor	Pre-clinical		Samanta *et al.*, 2006 Samanta *et al.*, 2009
	TG101209/HBC	JAK2 inhibitor	Pre-clinical		Samanta *et al.*, 2009; Traer *et al.*, 2012
	CYT387	JAK1/2 inhibitor	Pre-clinical		Traer *et al.*, 2012
	ONO44580	Dual JAK2 and ABL inhibitor	Preclinical		Samanta *et al.*, 2010
Hedgehog	Cyclopamine	Smoothened inhibitor	Pre-clinical		Zhao *et al.*, 2009; Dierks *et al.*, 2008
	LDE 225	Smoothened inhibitor	Phase I	NCT01456676	Irvine *et al.*, 2009; Zhang *et al.*, 2010
ALOX5	Zileuton	ALOX5 inhibitor	Phase I	NCT01130688	Chen *et al.*, 2009
PML	Arsenic trioxide	PML inhibitor	Phase I Phase II	NCT01397734 NCT00250042	Ito *et al.*, 2008; Goussetis *et al.*, 2012
	Arsenic sulphide	PML inhibitor	Pre-clinical		Zhang *et al.*, 2009
PP2A	FTY20	PP2A activator/SIP inhibitor	Pre-clinical		Neviani *et al.*, 2007; Oaks *et al.*, 2009; Neviani *et al.*, 2010
	OP449	SET inhibitor	Pre-clinical		Agarwal *et al.*, 2011;
PUFA	15d-PGJ_2/Δ^{12}-PGJ_3	Unknown	Pre-clinical		Hassane *et al.*, 2008; Hegde *et al.*, 2011
Nuclear export	KPT-185/KPT-207	CRM1 inhibitor	Pre-clinical		Walker *et al.*, 2012

Nuclear export inhibitors

Increased expression of nucleocytoplasmic shuttling heterogeneous nuclear ribonucleoproteins (hnRNPs) A1, E2 and K are critical in regulating proliferation and survival of Philadelphia positive progenitor cells. CRM1 is a karyopherin that controls nuclear export of hnRNPs; therefore, targeting CRM1 has therapeutic potential.

CRM1 plays an important role in the active transport of numerous cargo proteins, including transcription factors, tumour suppressors and cell cycle regulators. Deregulated oncogenic pathways, such as BCR-ABL signalling, have been shown to be associated with post-transcriptional modifications of tumour suppressor proteins, promoting their nuclear export through CRM1 (Keeshan *et al*., 2003). It seems intuitive therefore that prevention of nuclear export of tumour suppressor proteins has potential as a therapeutic modality in CML.

Until recently, development of CRM1 inhibitors has been hampered by their incumbent toxicity. The most well known of the CRM1 inhibitors is leptomycin B, a CRM1 inhibitor that is effective against a broad range of cancer cell lines *in vitro*, but is poorly tolerated *in vivo*. Novel oral small-molecule selective inhibitors of nuclear export (KPT-SINEs), which specifically block CRM1, have been developed (Karyopharm Therapeutics, Natick, MA, USA). These have shown activity in several cancer cell lines and most recently pre-clinical results report anti-leukaemic activity of KPT-SINEs (KPT-185 and 276) *in vitro* and *in vivo* in AML (Ranganathan *et al*., 2012).

Walker *et al*. (2012) have recently published data showing the impact of CRM1 inhibition on survival of BCR-ABL+ cells. Mouse BCR-ABL+ cells were exposed to CRM1 inhibitors KPT-185 and KPT-207 (Karyopharm Therapeutics). These decreased leukaemic cell viability with no effect on non-transformed cells. Their data suggest that CRM1 inhibition curbs survival of BCR-ABL+ cells through the prevention of export of hnRNPs from the nucleus of CML progenitor cells. These are critical for the preservation of proliferation signals triggered by the BCR-ABL oncogene. Restoration of PP2A through CRM1 inhibition was seen, highlighting the role of this tumour suppressor protein in leukaemogenesis.

It seems likely that these novel molecules represent a valuable addition to molecular therapies for CML, with potential to target the CML stem cell and its progeny, and merit further investigation as a prospect for achieving the elusive medical cure.

Conclusions

TKI provides an effective treatment against the kinase activity of BCR-ABL in mature cells in CML patients but cannot target the quiescent stem cell population; therefore, it is clear that an alternative therapy is required to target this population. Targeting members of key pathways modulated in LSC in comparison to normal cells is an attractive therapy for CML and there are many research avenues being pursued (Figure 1; Table 1). Various compounds that target members in these pathways are showing promise, both as single agents and in combination with TKI. Drug development, biological research and clinical trials are progressing and the future treatment of CML looks positive.

Acknowledgements

A. S. is funded by a BBSRC PhD scholarship and A. L. L. is funded by a CRUK PhD scholarship. T. L. H. is supported by a CRUK programme grant.

Authorship

A. S., A. L. L. and T. L. H. wrote the paper.

Conflict of interest

T. L. H. is a member of the Glivec Advisory board (Novartis), member of the dasatinib advisory board (Bristol-Myers Squibb) and principal investigator for the CHOICES clinical trial.

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