EDUCATIONAL ARTICLE

What is New in Chronic Myeloid Leukaemia?

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Abstract

Chronic myeloid leukaemia is a relatively rare condition, though it has stimulated widespread interest as a consequence of both the stem cell basis and the success of rationally designed therapies. This review will outline some of the issues involving the aetiology of the disease and how this relates to current and future therapies.

Introduction

Chronic myeloid leukaemia (CML) is classified by the World Health Organisation as a myeloproliferative disorder (MPD), one member of a group of conditions including essential thrombocythaemia, polycythaemia vera and chronic idiopathic myelofibrosis. The MPD are all clonal disorders of the haemopoietic stem cell (HSC) characterised by abnormal myeloid (i.e. granulocyte, erythroid and megakaryocytic) proliferation. CML (also termed chronic myelogenous or granulocytic leukaemia) is usually more appropriately considered as a distinct entity as a consequence of the now well characterised molecular biology of this disease. CML was first recognised in 1845[1, 2] with the initial description of the characteristic Philadelphia chromosome published in 1960.[3] Following this the causative *bcr-abl* oncogene with its protein product were described in the 1980s.[4, 5] This stimulated the search for rationally designed therapies of which imatinib (IM, Glivec®, STI571, Novartis Pharma), described in 1998, has achieved the most widespread use.

CML accounts for approximately one-tenth of all new leukaemia diagnoses. The reported incidence in Scotland has varied little over the past decade at around 60 new cases per year, with a median age at diagnosis of 68 years.[6] This can be compared to the published estimates of incidence at 1-2 per 100000 population with a median age at diagnosis of 45-55 years.[7]

Pathogenesis

CML arises as a consequence of a reciprocal translocation between the long arms of chromosomes 9 and 22 (t (9; 22)) in an HSC. The shortened form of chromosome 22 is named the Philadelphia chromosome (Ph), after the city in which it was discovered. The only accepted causative insult from which this rare mutational event may occur is exposure to high levels of radiation, such as in survivors of the Hiroshima bomb and Chernobyl clean-up workers.[8,9] The majority of cases diagnosed worldwide are thought to arise spontaneously with no clear evidence for environmental triggers. The t(9;22) translocates the proto-oncogene *abl* from chromosome 9 to 22 forming a chimeric oncogene *bcr-abl*. This gene is responsible for the production of Bcr-Abl, an oncoprotein with constitutive tyrosine kinase activity. Bcr-Abl confers proliferative and anti-apoptotic properties to the cell and is responsible for the pathogenesis of the disease.[10]

The HSC carrying t(9;22) (Ph+HSC) is capable of performing the normal HSC functions of self renewal (allowing perpetuation of the haemopoiesis) and differentiation to committed progeny, seen as mature circulating cells – all of which carry the cytogenetic abnormality and fusion oncogene. In the normal HSC pool, the majority of cells are quiescent entering cell cycle only once every 1-3 months.[11] This is in contrast with the deregulated Ph+HSC population where the majority of cells are in cycle at any one time. However, a quiescent diseased population remains, constituting approximately 0.5% of the affected HSC population.[12,13] This represents a potential reservoir of disease in a cell group that by virtue of their inactivity may be less susceptible to conventional therapy

The stem cell basis of CML has become a paradigm for the mechanism of disease in a number of other malignancies. These include acute myeloid leukaemia (AML), and non-haematological malignancies including tumours of the central nervous system and breast - all of which may arise of a consequence of deregulated stem cell activity.[14] However, CML is the only malignancy for which a specific causative cytogenetic abnormality has been identified.

Table I The definitions of accelerated phase and blast crisis as defined by major trials [20,35,36,51]. The diagnosis of chronic phase assumes the absence of any of the above criteria. *other than liver or spleen involvement † additional chromosomal abnormalities in Ph+ cells excluding variant Ph chromosome, loss of Y or constitutional abnormalities.

Accelerated Phase	Blast Crisis
Blast cells in blood or marrow 15-29%	Blast cells in blood or marrow ≥30%
Blast cells + promyelocytes in blood or marrow >30% (blasts <30%) Basophils in blood ≥20%	Extramedullary disease*
platelets <100 or >800x10^{9}/L (unrelated to therapy) clonal evolution†	

Clinical Features

The majority of patients diagnosed with CML are asymptomatic, with the diagnosis an unexpected outcome of a routine full blood count. The disease is divided into 3 recognised phases - chronic phase (CP), accelerated phase (AP) and blast crisis (BC) (Table I). At diagnosis patients are commonly in CP, a state characterised by leucocytosis and hepatosplenomegaly arising as a consequence of increased granulopoeisis and leukaemic infiltration. It has been shown that the presence of Bcr-Abl not only confers survival benefit, but influences the cell-stromal interactions within the bone marrow environment, leading to the release of more primitive cells into the circulation.[7] This is reflected in the characteristic blood film of a patient with CML as an elevated white cell count, with a shift in the myeloid line from mature to immature precursors ('left shift'). There are also typically an increased number of basophils and eosinophils. The bone marrow appearance is also typical with hypercellularity, a reversal of the normal ratio of erythroid to myeloid cells and a predominance of less mature forms. Untreated, CP will last a number of years and so it is suggested that the survival advantage of the leukaemic line is subtle relative to more aggressive diseases such as AML. The disease will then progress to BC, either directly or through an intermediate AP. Advanced stages (AP and BC) are characterised by a failure of maturation of the leukaemic precursors with the consequent disease resembling acute myeloid or lymphoblastic leukaemia. With modern therapy the progression of CML appears to be significantly slowed, however when advanced disease does occur it responds poorly to chemotherapy with the median survival measured in months.

Initial assessment for the patient newly diagnosed with CML includes a medical history and clinical examination to assess performance status, spleen size and the presence or absence of extramedullary disease. We would recommend as baseline investigations peripheral blood including full blood count with an accurate white cell differential and Bcr-Abl levels, by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). A bone marrow aspirate is necessary, confirming disease phase by assessment of morphology and allowing cytogenetic examination to quantify the percentage presence of the Ph+ clone (at least 20 metaphases should be examined). A small number of patients with CML may be Ph-, with the formation of *bcr-abl* arising as a result of more complex chromosomal translocations. The diseased cells may have abnormalities in addition to Ph+, including deletions of the derivative chromosome 9 (del(der)9). This finding has been associated with a poorer prognosis, though with newer therapies may be of less significance.[15,16] The baseline investigations serve to enable the clinician to determine the patient's prognostic category. There are 2 scoring systems accepted for use, both of which require information from time of diagnosis: the Sokal score, utilising spleen size in centimetres below the costal margin, the blast cell % (from a white cell differential count) and the platelet count; and the Hasford score using the same information but adding the basophil and eosinophil %. The scoring systems allow assignment of the patient into one of high, intermediate and poor prognostic groups.

The presence of the *bcr-abl* fusion gene transcript provides a mechanism for diagnosis and monitoring of minimal residual disease in CML. Initially this was done qualitatively using polymerase chain reaction (PCR) to detect only the presence or absence of transcripts. The evolution of the technology now allows quantitative monitoring of Bcr-Abl levels by real time PCR (RT-PCR). Results are expressed as a ratio of Bcr-Abl/Abl transcripts. The *abl* control gene data will provide an assessment of the quality and quantity of RNA being examined, providing an experimental sensitivity for each sample.[17,18] It has been demonstrated that the fall in levels with therapy reflects a reduction in disease burden. A major molecular response (MMolR) is defined as a greater than 3 log reduction in Bcr-Abl levels from baseline (or <0.1% Bcr-Abl level if the level at diagnosis is 100%).[18] The test is however not standarised between laboratories and efforts are been made to correct this by use of an international standard, enabling patient groups to be compared more reliably when assessing therapeutic responses.[18] Classification of responses can be made based on the full blood count, Bcr-Abl ratio and bone marrow cytogenetics as seen in Table II.

Table II Definition of responses. % Ph+ is % of cells containing the Philadelphia chromosome on cytogenetic analysis of at least 20 bone marrow metaphases [18]. A major cytogenetic response includes those with both a partial and complete cytogenetic response.

Response	Criteria
Haematological response	
Complete	Platelets <450x10^{9}/L
	White cell count <10x10^{9}/L
	Normal white cell differential
	Absence of splenomegaly
Cytogenetic response	
Complete	0% Ph+
Partial	1-35% Ph+
Major*	≤35% Ph+
Minor	36-65% Ph+
Minimal	66-95%Ph+
None	>95% Ph+
Molecular response	
Complete	Undetectable transcript
Major	≤0.1 Bcr/Abl control gene ratio

The current recommendation is that patients on therapy are monitored by Bcr-Abl measurements taken from peripheral blood every 3 months. A bone marrow aspirate is required to assess for cytogenetic responses, though the frequency of routine testing in the absence of evidence of altered disease activity is the subject of some debate. Standard guidelines recommend cytogenetic testing every 6 months until a complete cytogenetic response (CCyR) is achieved and hence annually.[19] There is no evidence that Bcr-Abl testing on marrow aspirate offers any more information than results derived from peripheral blood and the low levels of variation may lead to confusion in interpreting results.[20] We would recommend that a consistent approach to testing is adopted, allowing more reliable comparison of sequential results. There is prognostic significance in the serial monitoring of patient responses to therapy, in particular the response to IM.

Disease Therapy

Imatinib (IM)

The recommended standard first line therapy for patients with CML in CP is IM. This drug was shown to be superior to interferon-alpha (IFN-A) and cytosine arabinoside in the randomised prospective IRIS trial with initial data published in 2003. When adopted as initial therapy for those newly diagnosed with CP CML, IM led to a major improvement in outcome, as assessed by haematological, cytogenetic, molecular responses, progression free survival, side effects and quality of life.[21] Recent updated long term data generated from this trial reveals that 82% of patients achieve a CCyR with some late responses occurring in patients after 18 months treatment.[22] Early cytogenetic responses to IM are associated with improved outcome and can predict later response. Those with a partial cytogenetic response (PCyR) at 3, 6 or 12 months have a 90%, 80% or 50% chance respectively of achieving a complete cytogenetic response (CCyR) at 2 years. The benefit of achieving a major cytogenetic response (CCyR or PCyR) by 12 months is illustrated by recent data showing a 96% freedom from progression to advanced stages in this group, as compared to 81% in those with less than a partial response.[22]

Despite the impressive responses seen with IM there are some concerns about the long term efficacy. These have basis in the phenomena of disease persistence and IM resistance. It is accepted that despite the marked fall in Bcr-Abl measurements in those treated with IM therapy, with the majority of patients demonstrating a MMolR, few patients (<4%) will achieve a complete molecular response (i.e. undetectable Bcr-Abl).[23] Various groups, including our team in Glasgow, have focused on the Ph+HSC as the source of this minimal residual disease. We have shown that the quiescent Ph+HSC is not susceptible to the apoptotic effects of IM, even at concentrations greater than would be found within a treated patient. We have also demonstrated an accumulation of these quiescent cells with IM exposure of peripheral blood samples taken from patients at diagnosis of CML.[24] This work is complemented by that of Bhatia et al who have shown that patients who have confirmed CCyR on IM maintain a population of functional Bcr-Abl+ HSC.[25] It is possible to reverse the quiescent state of these Ph+HSC and reconstitute disease, shown by experiments where selected non-cycling diseased cells are transplanted into immunocompromised host mice, which then develop transplanted leukaemia.[12] This disease recrudescence is modeled in patients who discontinue IM therapy having achieved apparent disease control. The majority of patients rapidly relapse, though usually respond again to IM therapy.[26, 27, 28]

Resistance to IM is now a well recognised phenomenon. This may be primary, the failure of a patient to achieve a significant haematological or cytogenetic response, or secondary, manifest as reemergence of disease following an initial response. Resistance may occur in all phases of CML though is more common in AP and BC, where it may reflect the presence of additional cytogenetic abnormalities characteristic of advanced disease. Resistance may stem from: Bcr-Abl dependent mechanisms, such as point mutations in Bcr-Abl affecting IM binding, or amplification of the *bcr-abl* oncogene; and Bcr-Abl independent mechanisms, such as altered influx or

export of drug from the cell or sequestration of IM by plasma proteins.[29]

IM binds to the inactive form of the Abl kinase and once bound maintains the enzyme in an inert state by blocking phosphorylation, a prerequisite for activation.[30] The most common mechanism of resistance identified in patients treated with IM develops from mutations occurring within the Abl kinase domain of Bcr-Abl.[29] Cells will then no longer be susceptible to the antiproliferative and proapoptotic effects of the drug. This will enable the resistant clone to multiply under selective pressure, which may be reflected in disease recrudescence after a period of apparent control, with rising Bcr-Abl levels.

Altered drug export from cells is another well described mechanism of resistance to therapy in both solid organ and haematological malignancies. This forms the basis of the multidrug resistance (MDR) phenotype. MDR is the simultaneous development of resistance to more than one therapeutic agent and since it is not specific to the drug target, can concurrently affect drugs with different mechanisms of action. The gene MDR1 (also known as ABCB1) encodes P-glycoprotein (Pgp) a protein serving as a drug efflux pump. IM is a substrate of this transporter and it has been shown in cell lines that increased expression of Pgp correlates with IM-resistance.[31] It has also been seen using CML samples from patients resistant to IM, that IM-sensitivity may be restored with use of Pgp inhibitors *in vitro*.[32] This demonstrates the importance of identifying such transporters, as inhibition of their activity may act as a mechanism to overcome resistance and enhance effective intracellular drug concentration. Other transporters which may be involved in IM transport include ABCG2 (a drug exporter)[33,34] and Oct1 (involved in drug uptake).[17]

Newer Tyrosine Kinase Inhibitors

The qualified success of IM has stimulated the search for more effective tyrosine kinase inhibitors. Nilotinib (AMN107, Novartis) has been designed based on the molecular framework of IM, though with adjustments to the molecular structure which allow a better topographical fit with the target Bcr-Abl molecule. Initial *in vitro* work has demonstrated the increased potency of this drug for inhibiting proliferation of Bcr-Abl+ cells.[35] This has been carried forward into phase 1 trials where Nilotinib has been shown to be effective in those intolerant of, or resistant to IM. Responses were gained in patients with all phases of CML, though as with IM, significant responses were achieved more commonly in those with CP CML.[36] Dasatinib (BMS-354825, Bristol-Meyer Squibb) is dual Src- and Abl- kinase inhibitor which has also been shown *in vitro* to have potent effects on Bcr-Abl+ cell lines. Phase 1 trial data, published concurrently with that of nilotinib, demonstrates the effectiveness of this drug in the IM-resistant or intolerant population of CML patients in all phases of the disease. Again responses are more frequently seen in those with CP disease.[37] Despite this promising data, as with IM the problem of disease resistance and persistence may remain. Data produced from cell line studies and phase 1 trials show that neither nilotinib nor dasatinib has activity against the T315I mutation of Bcr-Abl. We have also shown that dasatinib, which demonstrates potent antiproliferative activity with enhanced cell kill in dividing Bcr-Abl+ cells, does not appear to eradicate the quiescent Ph+HSC population.[34] Similar data have been produced using nilotinib.[38]

It is hoped that both these drugs will obtain licenses for UK use in the near future. They will be useful additions to the treatment options available for patients, though it is not thought that initially they will replace IM as first line therapy. There will also be a small group of patients with the T315I mutation who will not respond to either therapy. The answer for these patients may be in the future development of drugs aimed at other targets in the CML cell such as farnesyl transferase inhibitors, proteasome inhibitors, heat shock protein 90 inhibitors and histone deacetylase inhibitors all of which are under investigations either as single agent or combination therapies.[38, 39, 40, 41, 42, 43] Of particular interest is research using an aurora kinase inhibitor with *in vitro* activity against cells expressing the T315I mutation.[44] The potential of future targeted therapies may ultimately be dependent on their ability to eliminate the Ph+HSC. A phase 1 trial which has completed recruitment in Glasgow is the granulocyte colony stimulating factor (G-CSF) and IM intermittently (GIMI) trial. This trial was designed following *in vitro* work using CD34+ selected populations derived from the bone marrow of patients newly diagnosed with CML. Exposure of these cells to pulses of G-CSF with during times of IM interruption appeared to significantly reduce the frequency of quiescent Ph+HSC detected. An explanation for this may be that the quiescent cells were stimulated by G-CSF to proliferate and so were rendered susceptible to the effects of IM.[38]

Bone Marrow Transplant

Haemopoietic stem cell transplantation (HSCT) is currently considered the only cure for CML. However it is limited in application by donor availability and the toxicity of conditioning regimes. The use of HSCT in CML has declined recently and a likely explanation for this is the advent of IM.[19] Despite the morbidity and mortality associated with standard myeloablative HSCT, it remains an important treatment option for those with IM intolerance or resistance.[19] HSCT with reduced intensity conditioning (RI-HSCT) is now an established treatment modality for a variety of different haematological malignancies. The procedure differs from standard myeloablative HSCT in the chemotherapy received by the recipient prior to transplantation. The regimes used are significantly less toxic and are therefore appropriate for a broader patient age range and performance status. Published data confirm that patients have shorter in patient stays, and engraft sooner with reduced transplant-related mortality.[45]

Relapse of CML post transplant is a recognised problem thought to occur in between 16 and 33% of patients.[45, 46] Donor lymphocyte infusion (DLI) has been shown to be particularly effective in eradicating CML post transplant with responses seen in 70-80% of patients.[47, 48] DLI may be given as a response to high or rising Bcr-Abl measurements or the presence of donor/host chimerism (indicating the likely presence of residual diseased host cells). DLI is associated with a risk of graft versus host disease (GvHD), a potentially significant accompaniment to the desired graft versus leukaemia (GvL) effect and marrow aplasia. The estimation of risk varies and may be minimised by the use of regimes involving a stepwise increase in cell dose given at set intervals.

There is also some controversy surrounding the use of IM in association with HSCT. IM use prior to transplant appears to be safe and does not appear to adversely affect outcome,[49] however the need for IM following transplant is less clear. IM is effective in the context of controlling relapsed disease of all phases,[50, 51, 52] though it is not known if it is routinely necessary. It is our view that the graft versus leukaemia effect of the RI-HSCT and subsequent DLI will enable disease eradication, however some would claim that maximal control with minimal risk of disease progression requires the sustained use of IM. This dilemma will hopefully be addressed following publication of trials currently in progress.

Conclusions

CML is a rare disease but one which has become a paradigm for the stem cell basis of a number of malignancies. The treatment of IM has also been a major success in rational drug design. Despite recent advances, there are a number of dilemmas remaining for the clinician treating and monitoring patients with CML.

IM remains the standard first line therapy for CML in CP, with many patients in the advanced stages of disease also responding to this treatment. The problems of IM-resistance and minimal residual disease remain. This contributes to the sustained risk of disease progression and prevents safe interruption of therapy. The mechanism of resistance may result in a disease that is also insensitive to the more potent therapies currently in trial, as seen with the T315I mutant. The options for such patients are limited. For this reason efforts continue to eradicate persistent disease. This may be with the use of next generation tyrosine kinase inhibitors, novel compounds with alternative targets or by the use of combinations of existing therapies.

Despite the decline in popularity of HSCT in CML it remains a valuable option. Those intolerant or failing to respond to IM require HSCT to enable long term disease control or cure. The role for RI-SCT is not yet clearly defined. It may be that patients with a matched donor who demonstrate features carrying risk of disease progression should be offered transplant. Should RI-SCT be offered up front to all patients with matched donors? This could cure their disease without the need for lifelong therapy with IM. The morbidity and mortality of the procedure with the likely subsequent need for DLI and the long term relapse risk require consideration and so this is currently considered an option for selected patients in experienced centres only.

CML is a paradigm for stem cell based disease and IM a successful example of rational drug design. The unique molecular basis of this disease will undoubtedly fuel further research with the aim of achieving a cure without the need for lifelong therapy.

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