Minimal Residual Disease and Discontinuation of Therapy in Chronic Myeloid Leukemia: Can We Aim at a Cure?

Junia V. Melo[1] and David M. Ross[2]

[1]Directorate of Haematology, SA Pathology, and Centre for Cancer Biology, University of Adelaide, Adelaide, Australia; and [2]Directorate of Haematology, SA Pathology, Flinders University and Medical Centre and School of Medicine, University of Adelaide, Adelaide, Australia

Patients with chronic myeloid leukemia (CML) who have achieved a complete molecular response (CMR) defined by no detectable *BCR-ABL* mRNA on imatinib (IM) treatment often ask whether it is necessary for treatment to continue. We now know that approximately 40% of patients with a stable CMR for at least 2 years are able to stop IM treatment and remain in molecular remission for at least 2 years. This exciting observation has raised hopes that many patients can be cured of CML without the need for transplantation and its attendant risks. One might argue that for many patients maintenance therapy with IM or an alternative kinase inhibitor is so well tolerated that there is no imperative to stop treatment; however, chronic medical therapy may be associated with impaired quality of life and reduced compliance. Inferences about the biology of CML in patients responding to kinase inhibitors can be drawn from clinical experience, molecular monitoring data, and experimental observations. We summarize this information herein, and propose 3 possible pathways to "cure" of CML by kinase inhibitors: stem-cell depletion, stem-cell exhaustion, and immunological control.

Introduction

Professor John Goldman suggested years ago that if a treatment is sufficient to prevent the progression of chronic myeloid leukemia (CML) to the advanced phase and to prevent the emergence of resistance, then the treatment can provide an "operational cure."[1] Effectively, CML responsive to imatinib (IM) might become a chronic disease requiring maintenance treatment, just as one might treat hypertension. Our own personal experience of counseling patients about IM cessation in a clinical trial suggests that some patients are comfortable with this view of their disease and feel secure on IM. However, if tyrosine kinase inhibitor (TKI) therapy is required indefinitely to maintain the operational cure of CML, then this ongoing drug exposure raises its own problems: there is a financial cost to the individual and the community, there may be chronic toxicity, and as long as there is residual disease there is at least a theoretical risk of emerging resistance.

With 5 years of follow-up < 10% of patients in the IRIS study stopped IM because of intolerable toxicity.[2] Rates of discontinuation for first-line nilotinib and dasatinib are similar at ~5% in the first year or so.[3,4] This is not to say that most patients have no significant toxicity. Chronic grade 1 toxicities such as diarrhea may significantly impair quality of life, and on occasion some patients choose not to take IM to avoid such toxicities.[5] Noncompliance has been identified as the single most important risk factor for loss of complete cytogenetic response in long-term IM-treated patients.[6] A recent update of progression-free survival in the IRIS study shows that there may still be rare patients who progress to the accelerated phase or to blast crisis after years of IM treatment,[7] and long-term compliance could be an important factor in durable disease control.

The potential for late-emerging toxicities remains unknown, given that our experience with TKI therapy spans little more than a decade, and young patients with CML may be facing many decades of exposure to an ABL kinase inhibitor. Potential concerns about cardiotoxicity with IM[8] seem not to have been borne out in clinical experience to date, but other side effects may become important in the longer term, including impaired glucose tolerance with nilotinib[3] and pleural effusion with dasatinib.[4] While such toxicities may be manageable, they impose an additional burden of morbidity.

In recent epidemiological data, the survival of younger patients treated with IM is within 10%-20% of the life expectancy of age-matched peers.[9] It is inevitable that patients should pursue other goals, such as parenthood. Patients in their reproductive years may be concerned about impaired fertility and the risk of teratogenesis. Although some women have had normal babies after conceiving on IM, in others, serious birth defects have been reported.[10] For some young women with CML the question of whether it is safe to stop IM for the duration of conception and pregnancy is as important as whether the disease is ever cured.

For all of these reasons we feel that an operational cure is only a partial success, and our ultimate aim should be to give the patient long-term disease control without the need for ongoing treatment.

ASCT

There already exists a curative treatment for CML. Allogeneic stem-cell transplantation (ASCT) in carefully selected younger patients may have an overall survival rate similar to that of IM-treated patients.[11] However, considering the average age of CML patients at diagnosis, the availability of a well-matched sibling donor, and the variability in outcome according to transplantation center, it is clear that this option applies only to a small minority of CML patients. The potential for chronic GVHD and complications related to myeloablative transplantation conditioning regimens, such as cardiopulmonary disease and an increased risk of secondary malignancy, may reduce quality of life and overall survival in

allografted patients. Several lines of evidence indicate that the curative potential of ASCT lies in an immunological GVL effect. If it were possible to harness this benefit by immunological manipulation without the toxicities of allografting this would be a major advance.

Very late relapses (after more than a decade) may occur even in patients allografted in the first chronic phase of CML.[12] A stable complete molecular response (CMR) is associated with a much lower risk of relapse after allograft, but even in this population, rare relapses are seen after more than 5 years.[13] These observations have implications for how we interpret the outcome of TKI cessation studies, which currently have much shorter follow-up.

Cessation of therapy

Interferon (IFN), with or without cytarabine, was the best drug therapy for CML before the introduction of IM. Approximately 1/4 of patients treated with IFN achieve a complete cytogenetic response, and only a small minority of patients will achieve a CMR.[14] Patients who stopped IFN after achieving a deep molecular response have been reported in stable minimal residual disease (MRD) for more than 9 years[15,16] and, much more rarely, in a stable CMR.[17]

The interim results of 2 studies of IM cessation have now been reported. The French STIM trial enrolled 100 patients with a stable CMR for at least 2 years.[18] Molecular relapse occurred in ~ 60% of patients after a median interval of 4 months. Relapses after 6 months were uncommon, but occurred as late as 19 months after stopping IM. The only patient characteristic that proved useful as a predictor of relapse risk was Sokal score: the probability of stable CMR was 54% in the low-risk group versus only 13% in patients with a high-risk score. The majority of relapses occurred only at a molecular level, and resumption of IM treatment rapidly restored CMR. The Australasian Leukaemia & Lymphoma Group (ALLG) CML8 study of imatinib cessation in CMR followed a similar design to the STIM trial, and has shown almost identical results in a smaller number of patients. At last analysis, the relapse rate was 60% (D.M. Ross and T.P. Hughes, personal communication). Given the potential for variability in molecular monitoring methods, it is reassuring to see comparable results from 2 independent investigations. In both studies the resumption of IM treatment resulted in a reduction of MRD, with most patients regaining a stable CMR within 6 months.

There have been no studies so far of therapy withdrawal in patients receiving newer ABL kinase inhibitors. We are, however, aware of a small number of patients who have been treated with either nilotinib or dasatinib after imatinib intolerance[19] or failure[20] and who stopped the TKI in CMR. Some of these patients remain in a stable remission with follow-up of up to 2 years. The number of such cases is currently too small to know whether the relapse rate after cessation of a second-generation inhibitor is different from that seen after stopping IM. With the increasing up-front use of these more potent ABL kinase inhibitors, it is likely to be only a short time before clinical trials are performed to answer this question.

What determines whether a patient will relapse when therapy is withdrawn? Relapse must arise from a pool of residual leukemic cells that are resistant to therapy. Conversely, drug-free remission might indicate eradication of the leukemic clone. In the ALLG CML8 study we examined this question using a highly sensitive patient-specific PCR for genomic *BCR-ABL* DNA.[21] This method has the advantage that problems of nonspecificity or contamination

are eliminated by virtue of the variability in the *BCR-ABL* fusion sequence.[21-23] This study showed that most patients who remained in CMR after stopping IM had circulating cells from the original CML clone. The level of MRD in these patients was ~ 1-2 log below the limit of detection of conventional real-time quantitative PCR (RQ-PCR). Thirteen of 17 patients had detectable *BCR-ABL* DNA at study entry, but only 8 of these patients relapsed (62%). Although this is the only study to examine highly sensitive MRD analysis in the context of IM cessation, at least 3 other studies have demonstrated the existence of residual leukemic cells in patients with a stable CMR.[22,24,25]

In the remainder of this article, 3 conceptual models for the achievement of a prolonged drug-free remission in CML are presented (Figure 1). The models are not mutually exclusive, and different pathways to drug-free remission might apply in different patients. Clinical implications for each of these models are also discussed.

Stem-cell depletion

Approximately 60% of patients in CMR are destined to relapse when IM is stopped. In these patients, there must be a population of CML precursors that is resistant to therapy. Analysis of RQ-PCR data from IM-treated CML patients reveals a biphasic response.[26] There is a rapid initial drop in the level of *BCR-ABL*, which is thought to reflect the clearance of mature CML progeny that are sensitive to IM. There follows a second phase with a shallow gradient, which is thought to reflect the gradual depletion of the less-sensitive CML granulocyte-macrophage precursor pool[27] which is relatively resistant to IM,[28,29] and in which the cellular events leading to blastic transformation are thought to occur.[30] The slow depletion of CML progenitors during IM treatment may reflect apoptosis that is dependent on cell cycling. In a kinetic model of cell-cycle–dependent stem-cell depletion, it was predicted that the disease could be eradicated by prolonged IM treatment.[27] Beyond the second year of treatment, the average reduction in *BCR-ABL* mRNA was < 0.5 log per year.[27,31] A conservative estimate of the number of residual CML cells in CMR would be ~ 10^6 cells, and simple extrapolation would lead us to predict that few patients would eradicate their leukemic clone within 10 years of first achieving CMR.

The available evidence from studies of primary CML stem cells in vitro strongly suggests that the most primitive compartment of quiescent leukemic stem cells is resistant not only to IM, but also to the more potent second-generation ABL kinase inhibitors,[32] and this should mean that ongoing therapy is necessary to suppress the CML clone. This pessimistic prediction is at odds with the observed relapse rate when IM is stopped after as little as 2 years in CMR. The absence of early relapse does not indicate that these patients are cured, but it is inconsistent with unchecked proliferation of CML from a level that is only 1-2 log below the limit of detection of RQ-PCR. Furthermore, the duration of CMR before IM cessation was not significant as a predictor of relapse risk in the French study, although total duration of TKI therapy and Sokal risk score were.[18] A plausible explanation for the correlation between Sokal score and relapse risk is that the clinical risk score is a surrogate for the sensitivity of CML progenitors to IM. If this is the case, then stable CMR after IM withdrawal might be explained by more rapid depletion of CML stem cells in a subset of patients.

While we have so far considered the level of MRD in CMR based on extrapolation from the kinetics of response, it is also possible to
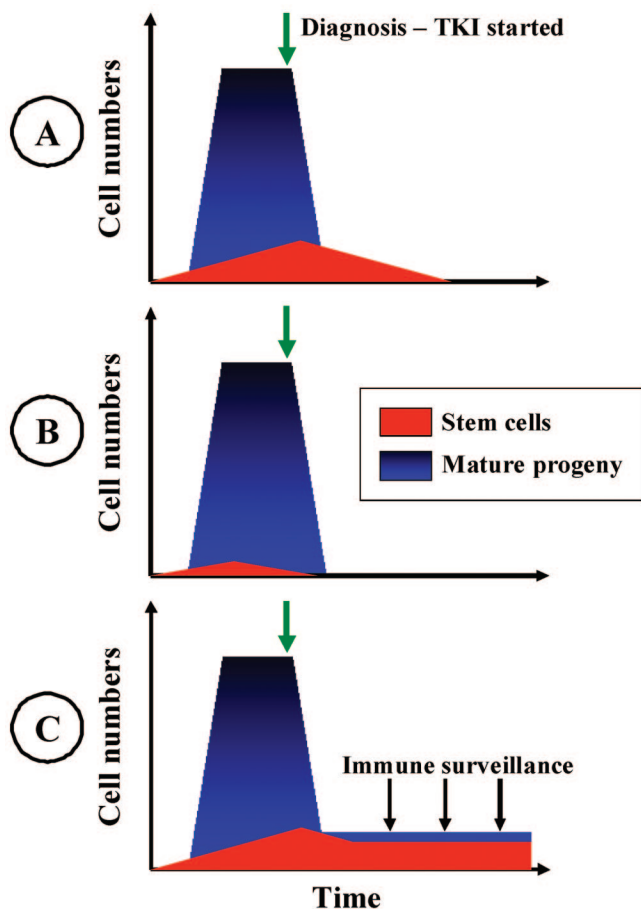
Figure 1. Three hypothetical models of operational cure of CML. The models are not mutually exclusive. Different models may pertain in different individuals. (A) Stem-cell depletion. The progressive depletion of immature CML cells over years of continued therapy is shown. The risk of relapse on TKI cessation is related to the duration of therapy and the intrinsic sensitivity of CML stem and early precursor cells. (B) Stem-cell exhaustion. The CML stem-cell pool is relatively small and, due to stochastic events that direct self-renewal or proliferation, this pool of cells may become extinct before diagnosis or early in therapy. The risk of relapse upon TKI cessation is related to the depletion of committed CML progenitors and progeny. (C) Immunological control. A reduction in the level of MRD by TKI therapy is sufficient to overcome T-cell anergy and enables the emergence of an autologous immunological response that suppresses, but may not eradicate, the CML clone. The risk of relapse on TKI cessation is dependent on the functional immune response and the intrinsic immunogenicity of the CML cells.

extrapolate from the kinetics of relapse. Modeling of relapsing CML using RQ-PCR data predicts a rapid increase in *BCR-ABL* with a doubling time of about 2 weeks, which is consistent with relapse from a pool of committed myeloid precursors rather than from slow-cycling stem cells.[26,27] This model fits with the clinical observation that patients who relapse usually do so within several months of stopping IM, and indicates that, for these patients, IM treatment has not effectively targeted the stem-cell compartment.

In both the French STIM study and the Australian CML8 study, occasional patients have been observed with late relapses more than a year after stopping imatinib. One hypothesis to explain this observation is that there is a change in the proliferation rate of the residual CML cells so that their doubling time is significantly prolonged. This hypothesis is not supported by experimental data showing that CML cells that survive TKI therapy can proliferate efficiently when the TKI is withdrawn.[33] However, it remains possible that prolonged TKI exposure in vivo might modify the interaction between the CML cells and the stroma or microenvironment to impair the proliferative potential of residual CML cells.

What of those patients who have evidence of MRD based on the detection of genomic *BCR-ABL* DNA while in CMR without therapy? These data would seem to indicate that stable CMR is, in most cases, not dependent on complete eradication of the leukemic clone. This is an important objection to the stem-cell depletion model of cure. One possible explanation is that the residual leukemic cells measured are mature progeny with no self-renewal potential—that is, that the cells in the peripheral blood arise from a population that is doomed to proliferate for a short time and then become extinct. A similar model has been proposed as an explanation for the detection of *BCR-ABL* in normal individuals.[34] Whether this hypothesis explains our observations in CML patients after IM withdrawal will be answerable with longer follow-up.

If the principal determinant of relapse risk is the depletion of CML stem cells by treatment, then there is a need for strategies to target these cells in at least 60% of patients. Because there is a range of biological pathways that typify CML stem cells, there is a similarly wide range of agents to target this population. CML stem cells share many properties with normal stem cells,[35] so it may be challenging to develop a therapy that is selective for leukemic stem cells with acceptable toxicity. Studies on primitive CML cells in vitro have shown multiple mechanisms of resistance to ABL kinase inhibitors,[28] including decreased intracellular uptake and retention of cytotoxic drugs and TKIs,[28] decreased expression of HLA costimulatory molecules and targets of adaptive immunity (eg, myeloid granule proteins), and resistance to apoptosis.[33,36] Treatment with ABL kinase inhibitors in vitro results in an increase in the fraction of quiescent cells that is highly resistant to TKI therapy.[32,37] IFN may induce the proliferation of hematopoietic stem cells,[38] and if this is also true of BCR-ABL+ stem cells, then it could mean that the combination of IFN with a TKI might then be more effective against quiescent CML stem cells. The combination of IM and IFN has been used successfully, with promising early molecular responses in 2 studies,[39,40] a result that has not been observed in 2 other trials[41,42] possibly due to high rates of intolerance or differences between IFN preparations. The combination of IFN and IM did not improve cytogenetic responses or progression-free survival in any of the 4 studies reported. The long-term effect on the rate of CMR and the potential for drug-free remission is unclear.

The phosphorylation status of BCR-ABL substrates depends on the balance between the activity of tyrosine kinases and protein phosphatases. Many substrates of BCR-ABL are dephosphorylated by protein phosphatase 2A (PP2A), and the activity of PP2A is decreased in CML and in various other hematologic malignancies.[43] The small molecule FTY720[44] and the plant derivative forskolin[43] both increase PP2A activity, providing a rationale for the combination of one of these agents with an ABL kinase inhibitor. There is preliminary evidence that self-renewal of CML stem cells is not dependent on BCR-ABL kinase activity. FTY720 treatment of quiescent BCR-ABL+ stem cells degraded β-catenin and induced apoptosis, with no effect on normal stem cells.[45,46] One of the most important pathways for self-renewal is the Wnt-β-catenin pathway. Inhibitors of Smo, a molecule in the Hedgehog pathway, have

stem-cell–targeting activity, and the combination of LDE 225 and nilotinib in vitro resulted in a decrease in CML stem cells in a transgenic mouse model.[47] Another Smo inhibitor, GDC449, has been used in patients with solid tumors with some response and acceptable toxicity.[48]

Another possible mechanism by which CML stem cells survive ABL kinase inhibition is autocrine or paracrine signaling through cytokine receptors. The addition of hematopoietic growth factors to the culture medium of primary CML cells exposed to dasatinib in vitro increases the survival of these cells.[49] Because many cytokines signal through the JAK-STAT pathway, the combination of a TKI with a JAK2 inhibitor might be beneficial to target MRD. Stromal interactions have also been proposed to protect stem cells in vivo, providing a rationale for disrupting marrow homing through the CXCR4 with the mobilizing agent plerixafor.[50] Other agents that have promise include farnesyl transferase inhibitors,[51] histone deacetylase inhibitors,[52] and proteasome inhibitors.[53] Choosing the best combination of stem-cell–targeting agent to move into clinical trials will depend on the balance of in vitro efficacy and clinical safety data.

Stem-cell exhaustion

An alternative hypothesis to explain eradication of the CML clone without the need for activity of ABL kinase inhibitors against CML stem cells is stem-cell exhaustion. It is believed that the numbers of stem cells or early progenitor cells are regulated by a balance between symmetric and asymmetric cell division. Symmetric division, in which the daughter cells are committed either to differentiation or to self-renewal must occur in order for the stem-cell pool to expand and markedly increase the output of mature progeny. In a large population of stem cells, regulators of hematopoiesis such as cytokines and stromal interactions will ensure that the proportion of cells that commits to self-renewal is sufficient to maintain long-term hematopoiesis. However, for an individual cell, it is possible that both progeny should commit to differentiation and that the "clone" would become extinct once its progeny complete differentiation and senesce. This may be one of the reasons for graft failure after autologous stem-cell transplantation with a marginal cell dose.[54] Using X chromosome inactivation patterns in women of various ages, it is possible to estimate the number of hematopoietic stem cells contributing to hematopoiesis throughout the human lifespan. Combining these observations with data from animal experiments, the number of true hematopoietic stem cells in a young adult was estimated at ∼ 10 000, and the critical number needed to ensure stable hematopoiesis after autologous stem-cell transplantation was ∼ 100.[54]

In a mathematical model of CML incorporating a stochastic process in which stem cells could undergo symmetric division and commit to differentiation, the CML stem-cell pool was already exhausted before most virtual patients reached diagnosis.[55] After commencement of IM treatment, the proportion of "patients" in whom the CML stem-cell pool became extinct increased progressively over time. Similar conclusions were reached by a second group using a stochastic model of myeloproliferative neoplasms in small animals in which hematopoiesis has been well-characterized.[56] The introduction of a single neoplastic stem cell in the model led to clonal predominance in only ∼ 15%-20% of simulations. Assumptions about normal hematopoietic stem cells may not always be applicable to CML stem cells. The presence of BCR-ABL in stem cells may slightly shift the balance between self-renewal, apoptosis, and proliferation to reduce the self-renewal potential of the CML stem

cells relative to normal stem cells.[35] Such a shift would increase the probability of spontaneous exhaustion of the CML stem-cell pool when small numbers are present.

If this hypothesis were correct, what implications would it have for our approach to the treatment of CML? It would mean that all patients could potentially be cured by prolonged suppression of the more mature CML cells. It is thought that the acquisition of new mutations in the granulocyte-macrophage precursor compartment is responsible for the acquisition of ectopic self-renewal capacity and transformation to blast crisis.[30] Therefore, perhaps these more mature progenitor cells should be our main targets for eradication. Numerous assumptions go into a stochastic model, and the possibility of spontaneous exhaustion of the CML clone remains for now a tantalizing hypothesis. Nevertheless, it provides us with a different perspective on CML stem cells and their significance as the ultimate targets of therapy.

Immunological control

In the ALLG CML8 study of IM withdrawal in CMR we showed that a stable level of MRD could be measured in patients who did not relapse.[21] Because cessation of TKI therapy commonly results in relapse within 6 months, it is possible that in those patients who do not relapse, there is ongoing suppression of the CML clone, potentially mediated by an autologous T-cell response. In contrast to stem-cell–targeted therapies, in which the target effect is almost impossible to measure, an immunological approach has the advantage that functional immune responses can be measured in the peripheral blood or BM, and might prove useful as surrogate markers of response.

The strongest evidence for immunological control of CML comes from ASCT, with responses to donor lymphocyte infusion highlighting the importance of T cells. In IFN-treated patients, there is an association between clinical response to IFN and the emergence of CTLs specific for myeloid-associated antigens.[57] Higher expression of the myeloid-associated antigen proteinase 3 was associated with more indolent disease,[58] suggesting that a more immunogenic CML clone is more effectively suppressed by the host immune system. If immunological control of MRD is important in determining relapse risk, what are the therapeutic implications? There are currently no data on CTL responses as a predictor of relapse risk after TKI withdrawal. Clonal T-cell proliferations occur in some dasatinib-treated patients and have been associated with a good response to therapy.[59] Similar CTL responses may occur during IM treatment,[60] and rare CTLs against CML-associated antigens can be found even in the blood of normal individuals.[61] Studies of CTL responses in CMR patients should help to answer this question, and might help to improve the selection of candidates for a trial of TKI withdrawal.

CTL responses against leukemia-associated antigens may emerge in response to a reduction in disease burden.[57,60] In CML, the reduction of the antigenic burden by TKI therapy may enable the recovery of a functional CTL response that had been rendered anergic. The potential importance of anergic CTL responses was shown in a mouse model of CML in which up-regulation of the receptor for a T-cell–costimulatory molecule, Programmed Cell Death-1, was associated with immune exhaustion and disease progression.[62] Pharmacological manipulation of costimulatory molecules involved in the CTL response could provide a novel therapeutic approach in CML.

One of several mechanisms by which IFN might contribute to control of CML is by increasing the expression of leukemia-associated antigens and augmenting the CTL response.[57,63] Burchert et al devised a rational approach to combination therapy with IFN and IM in which both drugs were given together to reduce disease burden, and then IFN monotherapy was used as maintenance treatment.[64] Leukemia-specific CTLs were observed more frequently after IM was stopped, and there was a suggestion that the loss of response was associated with failure to develop an immunological CTL response. An alternative approach that has been used to augment leukemia-specific CTLs is vaccination.[65,66]

Summary

Various strategies might improve the odds of drug-free remission of CML, and some of these have already been used in clinical trials. Given the excellent safety and tolerability of TKI therapy, the safety of any novel treatment for this patient group is of paramount importance. Any augmentation of TKI therapy must carry a risk of new, additional, or unexpected toxicity, and this risk must be balanced against the potential benefit. The same consideration of patient safety applies to a therapeutic test of TKI withdrawal. It is essential that careful monitoring is undertaken to detect relapse as early as possible so that effective TKI therapy may be reinstituted. It is therefore recommended that, at present, such approaches are only considered in the context of carefully designed, controlled clinical trials.

Sustained CMR after withdrawal of TKIs is a therapeutic success that many clinicians and scientists would not have anticipated when IM treatment was first introduced. By analogy with the treatment of HIV with antiretroviral drugs, it seemed likely that even a highly effective targeted treatment would need to be continued indefinitely to suppress the CML clone. Perhaps contrary to expectation, this is not always the case in chronic-phase CML. Early and deep response translates to long-term disease control with the potential for safe withdrawal of treatment in carefully selected patients. The challenges now are to identify those patients in whom treatment can safely be withdrawn, and to increase the number of patients eligible for withdrawal of therapy.

Disclosures

Conflict-of-interest disclosure: J.V.M. declares no competing financial interests. D.M.R. has received research funding and honoraria from Novartis Pharmaceuticals. Off-label drug use: None disclosed.

Correspondence

Professor Junia V. Melo, Department of Haematology, Centre for Cancer Biology, SA Pathology, Frome Road, Adelaide, SA 5000, Australia; Phone: 61-8-8222-3441; Fax: 61-8-8222-3139; e-mail: junia.melo@health.sa.gov.au.

References

1. Goldman J, Gordon M. Why do chronic myelogenous leukemia stem cells survive allogeneic stem cell transplantation or imatinib: does it really matter? *Leuk Lymphoma*. 2006;47:1-7.
2. Druker BJ, Guilhot F, O'Brien SG, et al. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *N Engl J Med*. 2006;355:2408-2417.
3. Saglio G, Kim DW, Issaragrisil S, et al. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. *N Engl J Med*. 2010;362:2251-2259.
4. Kantarjian H, Shah NP, Hochhaus A, et al. Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med*. 2010;362:2260-2270.
5. Eliasson L, Clifford S, Barber N, Marin D. Exploring chronic myeloid leukemia patients' reasons for not adhering to the oral anticancer drug imatinib as prescribed. *Leuk Res*. 2011;35:626-630.
6. Ibrahim AR, Eliasson L, Apperley JF, et al. Poor adherence is the main reason for loss of CCyR and imatinib failure for CML patients on long term therapy. *Blood*. 2011;117:3733-3736.
7. Deininger M, O'Brien S, Guilhot F, et al. International Randomized Study of Interferon vs STI571 (IRIS) 8-year follow up: sustained survival and low risk for progression or events in patients with newly diagnosed chronic myeloid leukemia in chronic phase (CML-CP) treated with imatinib [Abstract]. *Blood*. 2009;114:1126.
8. Kerkelä R, Grazette L, Yacobi R, et al. Cardiotoxicity of the cancer therapeutic agent imatinib mesylate. *Nat Med*. 2006;12:908-916.
9. Björkholm M, Ohm L, Eloranta S, et al. Success story of targeted therapy in chronic myeloid leukemia: a population-based study of patients diagnosed in sweden from 1973 to 2008. *J Clin Oncol*. 2011;29:2514-2520.
10. Apperley J. CML in pregnancy and childhood. *Best Pract Res Clin Haematol*. 2009;22:455-474.
11. Saussele S, Lauseker M, Gratwohl A, et al. Allogeneic hematopoietic stem cell transplantation (allo SCT) for chronic myeloid leukemia in the imatinib era: evaluation of its impact within a subgroup of the randomized German CML Study IV. *Blood*. 2010;115:1880-1885.
12. Gratwohl A, Brand R, Apperley J, et al. Allogeneic hematopoietic stem cell transplantation for chronic myeloid leukemia in Europe 2006: transplant activity, long-term data and current results. An analysis by the Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT). *Haematologica*. 2006;91:513-521.
13. Mughal TI, Yong A, Szydlo RM, et al. Molecular studies in patients with chronic myeloid leukaemia in remission 5 years after allogeneic stem cell transplant define the risk of subsequent relapse. *Br J Haematol*. 2001;115:569-574.
14. Guilhot F, Roy L, Guilhot J, Millot F. Interferon therapy in chronic myelogenous leukemia. *Hematol Oncol Clin North Am*. 2004;18:585-603.
15. Mahon FX, Delbrel X, Cony-Makhoul P, et al. Follow-up of complete cytogenetic remission in patients with chronic myeloid leukemia after cessation of interferon alfa. *J Clin Oncol*. 2002;20:214-220.
16. Verbeek W, Konig H, Boehm J, et al. Continuous complete hematological and cytogenetic remission with molecular minimal residual disease 9 years after discontinuation of interferon-alpha in a patient with Philadelphia chromosome-positive chronic myeloid leukemia. *Acta Haematol*. 2006;115:109-112.
17. Kantarjian HM, O'Brien S, Cortes JE, et al. Complete cytogenetic and molecular responses to interferon-alpha-based therapy for chronic myelogenous leukemia are associated with excellent long-term prognosis. *Cancer*. 2003;97:1033-1041.
18. Mahon FX, Rea D, Guilhot J, et al. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial. *Lancet Oncol*. 2010;11:1029-1035.
19. Rea D, Rousselot P, Nicolini F, et al. Cessation of dasatinib or nilotinib therapy in chronic-phase chronic myeloid leukaemia

American Society of Hematology

patients with sustained complete molecular responses [Abstract]. *Haematologica*. 2011;96:1014.

20. Ross DM, Bartley PA, Goyne J, Morley AA, Seymour JF, Grigg AP. Durable complete molecular remission of chronic myeloid leukemia following dasatinib cessation, despite adverse disease features [published online ahead of print August 9, 2011]. *Haematologica*. doi:10.3324/haematol.2011.048165.

21. Ross DM, Branford S, Seymour JF, et al. Patients with chronic myeloid leukemia who maintain a complete molecular response after stopping imatinib treatment have evidence of persistent leukemia by DNA PCR. *Leukemia*. 2010;24:1719-1724.

22. Sobrinho-Simões M, Wilczek V, Score J, Cross NC, Apperley JF, Melo JV. In search of the original leukemic clone in chronic myeloid leukemia patients in complete molecular remission after stem cell transplantation or imatinib. *Blood*. 2010;116: 1329-1335.

23. Bartley PA, Ross DM, Latham S, et al. Sensitive detection and quantification of minimal residual disease in chronic myeloid leukaemia using nested quantitative PCR for BCR-ABL DNA. *Int J Lab Hematol*. 2010;32:e222-228.

24. Goh HG, Lin M, Fukushima T, et al. Sensitive quantitation of minimal residual disease in chronic myeloid leukemia using nanofluidic digital polymerase chain reaction assay. *Leuk Lymphoma*. 2011;52:896-904.

25. Mahon F-X, Dulucq S, Guilhot F, et al. A more sensitive RQ-PCR to assess complete molecular remission does not allow the prediction of relapse after discontinuation of imatinib in chronic myeloid leukemia [Abstract]. *Blood*. 2010;116:2298.

26. Michor F, Hughes TP, Iwasa Y, et al. Dynamics of chronic myeloid leukaemia. *Nature*. 2005;435:1267-1270.

27. Roeder I, Horn M, Glauche I, Hochhaus A, Mueller MC, Loeffler M. Dynamic modeling of imatinib-treated chronic myeloid leukemia: functional insights and clinical implications. *Nat Med*. 2006;12:1181-1184.

28. Jiang X, Zhao Y, Smith C, et al. Chronic myeloid leukemia stem cells possess multiple unique features of resistance to BCR-ABL targeted therapies. *Leukemia*. 2007;21:926-935.

29. Graham SM, Jorgensen HG, Allan E, et al. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. *Blood*. 2002;99:319-325.

30. Jamieson CH, Ailles LE, Dylla SJ, et al. Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. *N Engl J Med*. 2004;351:657-667.

31. Branford S, Seymour JF, Grigg A, et al. BCR-ABL messenger RNA levels continue to decline in patients with chronic phase chronic myeloid leukemia treated with imatinib for more than 5 years and approximately half of all first-line treated patients have stable undetectable BCR-ABL using strict sensitivity criteria. *Clin Cancer Res*. 2007;13:7080-7085.

32. Copland M, Hamilton A, Elrick LJ, et al. Dasatinib (BMS-354825) targets an earlier progenitor population than imatinib in primary CML but does not eliminate the quiescent fraction. *Blood*. 2006;107:4532-4539.

33. Holyoake TL, Jiang X, Jorgensen HG, et al. Primitive quiescent leukemic cells from patients with chronic myeloid leukemia spontaneously initiate factor-independent growth in vitro in association with up-regulation of expression of interleukin-3. *Blood*. 2001;97:720-728.

34. Bose S, Deininger M, Gora-Tybor J, Goldman JM, Melo JV. The presence of typical and atypical BCR-ABL fusion genes in leukocytes of normal individuals: biologic significance and implications for the assessment of minimal residual disease. *Blood*. 1998;92:3362-3367.

35. Sloma I, Jiang X, Eaves AC, Eaves CJ. Insights into the stem cells of chronic myeloid leukemia. *Leukemia*. 2010;24:1823-1833.

36. Dorsey JF, Cunnick JM, Lanehart R, et al. Interleukin-3 protects Bcr-Abl-transformed hematopoietic progenitor cells from apoptosis induced by Bcr-Abl tyrosine kinase inhibitors. *Leukemia*. 2002;16:1589-1595.

37. Holyoake T, Jiang X, Eaves C, Eaves A. Isolation of a highly quiescent subpopulation of primitive leukemic cells in chronic myeloid leukemia. *Blood*. 1999;94:2056-2064.

38. Essers MA, Offner S, Blanco-Bose WE, et al. IFNalpha activates dormant haematopoietic stem cells in vivo. *Nature*. 2009;458:904-908.

39. Preudhomme C, Guilhot J, Nicolini FE, et al. Imatinib plus peginterferon alfa-2a in chronic myeloid leukemia. *N Engl J Med*. 2010;363:2511-2521.

40. Simonsson B, Gedde-Dahl T, Markevarn B, et al. Combination of pegylated interferon-{alpha}2b with imatinib increases molecular response rates in patients with low or intermediate risk chronic myeloid leukemia [published online ahead of print, June 17, 2011]. *Blood*. doi:10.1182/blood-2011-02-336685.

41. Cortes J, Quintas-Cardama A, Jones D, et al. Immune modulation of minimal residual disease in early chronic phase chronic myelogenous leukemia: a randomized trial of frontline high-dose imatinib mesylate with or without pegylated interferon alpha-2b and granulocyte-macrophage colony-stimulating factor. *Cancer*. 2011;117:572-580.

42. Hehlmann R, Lauseker M, Jung-Munkwitz S, et al. Tolerability-adapted imatinib 800 mg/d versus 400 mg/d versus 400 mg/d plus interferon-alpha in newly diagnosed chronic myeloid leukemia. *J Clin Oncol*. 2011;29:1634-1642.

43. Neviani P, Santhanam R, Trotta R, et al. The tumor suppressor PP2A is functionally inactivated in blast crisis CML through the inhibitory activity of the BCR/ABL-regulated SET protein. *Cancer Cell*. 2005;8:355-368.

44. Neviani P, Santhanam R, Oaks JJ, et al. FTY720, a new alternative for treating blast crisis chronic myelogenous leukemia and Philadelphia chromosome-positive acute lymphocytic leukemia. *J Clin Invest*. 2007;117:2408-2421.

45. Oaks J, Neviani P, Mukhopadhyay A, et al. FTY720 but not its immunosuppressive phosphorylated form FTY720-P exerts anti-leukemic activity towards Ph(+) and Ph(-) myeloproliferative disorders through reactivation of the PP2A tumor suppressor [Abstract]. *Blood*. 2009;114:3259.

46. Neviani P, Harb JG, Oaks J, et al. BCR-ABL1 kinase activity but not its expression is dispensable for Ph+ quiescent stem cell survival which depends on the PP2A-controlled Jak2 activation and is sensitive to FTY720 treatment [Abstract]. *Blood*. 2010;116:515.

47. Zhang B, Irvine D, Ho YW, et al. Inhibition of chronic myeloid leukemia stem cells by the combination of the hedgehog pathway inhibitor LDE225 with nilotinib [Abstract]. *Blood*. 2010;116:514.

48. LoRusso PM, Rudin CM, Reddy JC, et al. Phase I trial of hedgehog pathway inhibitor vismodegib (GDC-0449) in patients with refractory, locally advanced or metastatic solid tumors. *Clin Cancer Res*. 2011;17:2502-2511.

49. Hiwase DK, White DL, Powell JA, et al. Blocking cytokine signaling along with intense Bcr-Abl kinase inhibition induces apoptosis in primary CML progenitors. *Leukemia*. 2010;24:771-778.

50. Dillmann F, Veldwijk MR, Laufs S, et al. Plerixafor inhibits chemotaxis toward SDF-1 and CXCR4-mediated stroma contact in a dose-dependent manner resulting in increased susceptibility of BCR-ABL+ cell to Imatinib and Nilotinib. *Leuk Lymphoma*. 2009;50:1676-1686.

51. Copland M, Pellicano F, Richmond L, et al. BMS-214662 potently induces apoptosis of chronic myeloid leukemia stem and progenitor cells and synergizes with tyrosine kinase inhibitors. *Blood*. 2008;111:2843-2853.

52. Zhang B, Strauss AC, Chu S, et al. Effective targeting of quiescent chronic myelogenous leukemia stem cells by histone deacetylase inhibitors in combination with imatinib mesylate. *Cancer Cell*. 2010;17:427-442.

53. Yong AS, Keyvanfar K, Hensel N, et al. Primitive quiescent CD34+ cells in chronic myeloid leukemia are targeted by in vitro expanded natural killer cells, which are functionally enhanced by bortezomib. *Blood*. 2009;113:875-882.

54. Catlin SN, Busque L, Gale RE, Guttorp P, Abkowitz JL. The replication rate of human hematopoietic stem cells in vivo. *Blood*. 2011;117:4460-4466.

55. Lenaerts T, Pacheco JM, Traulsen A, Dingli D. Tyrosine kinase inhibitor therapy can cure chronic myeloid leukemia without hitting leukemic stem cells. *Haematologica*. 2010;95:900-907.

56. Catlin SN, Guttorp P, Abkowitz JL. The kinetics of clonal dominance in myeloproliferative disorders. *Blood*. 2005;106: 2688-2692.

57. Molldrem JJ, Lee PP, Wang C, et al. Evidence that specific T lymphocytes may participate in the elimination of chronic myelogenous leukemia. *Nat Med*. 2000;6:1018-1023.

58. Yong AS, Szydlo RM, Goldman JM, Apperley JF, Melo JV. Molecular profiling of CD34+ cells identifies low expression of CD7, along with high expression of proteinase 3 or elastase, as predictors of longer survival in patients with CML. *Blood*. 2006;107:205-212.

59. Mustjoki S, Ekblom M, Arstila TP, et al. Clonal expansion of T/NK-cells during tyrosine kinase inhibitor dasatinib therapy. *Leukemia*. 2009;23:1398-1405.

60. Chen CI, Maecker HT, Lee PP. Development and dynamics of robust T-cell responses to CML under imatinib treatment. *Blood*. 2008;111:5342-5349.

61. Butt NM, Rojas JM, Wang L, Christmas SE, Abu-Eisha HM, Clark RE. Circulating bcr-abl-specific CD8+ T cells in chronic myeloid leukemia patients and healthy subjects. *Haematologica*. 2005;90:1315-1323.

62. Mumprecht S, Schurch C, Schwaller J, Solenthaler M, Ochsenbein AF. Programmed death 1 signaling on chronic myeloid leukemia-specific T cells results in T-cell exhaustion and disease progression. *Blood*. 2009;114:1528-1536.

63. Burchert A, Wolfl S, Schmidt M, et al. Interferon-alpha, but not the ABL-kinase inhibitor imatinib (STI571), induces expression of myeloblastin and a specific T-cell response in chronic myeloid leukemia. *Blood*. 2003;101:259-264.

64. Burchert A, Muller MC, Kostrewa P, et al. Sustained molecular response with interferon alfa maintenance after induction therapy with imatinib plus interferon alfa in patients with chronic myeloid leukemia. *J Clin Oncol*. 2010;28:1429-1435.

65. Bocchia M, Gentili S, Abruzzese E, et al. Effect of a p210 multipeptide vaccine associated with imatinib or interferon in patients with chronic myeloid leukaemia and persistent residual disease: a multicentre observational trial. *Lancet*. 2005;365:657-662.

66. Rojas JM, Knight K, Wang L, Clark RE. Clinical evaluation of BCR-ABL peptide immunisation in chronic myeloid leukaemia: results of the EPIC study. *Leukemia*. 2007;21:2287-2295.