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What challenges remain in chronic myeloid leukemia research?

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In the last fifteen years, the advent of imatinib has opened a new era in the treatment of CML. The challenge now is to eradicate the disease. To do this, groundbreaking scientific and biological studies should lead to the development of new techniques that can eliminate the last leukemic cells. Some of the salient future possibilities are summarized here.

Initial choice of therapy

Imatinib was introduced in 1998 and has until now been the preferred first-line therapy for newly diagnosed CML patients. Unfortunately, approximately 30-35% of the patients who receive this drug do not respond optimally or its administration has to be interrupted because of side effects. Nilotinib and dasatinib have been used as a second-line therapy. Both these agents can induce complete cytogenetic responses (CCyR) in approximately 50% and major molecular remission (MMR) in 20-30% of imatinib resistant patients.[1,2] When these drugs were compared with imatinib as initial treatment in two randomized clinical trials,[3-5] the responses were faster and superior with deeper molecular responses than with imatinib. Moreover, nilotinib and dasatinib were active also against different kinase domain mutations, even though neither drug inhibited clones with the T315I mutation. In general, both these drugs have good tolerability, though nilotinib showed a higher incidence of gastrointestinal toxicity than imatinib, while dasatinib induced more pleural effusions and more myelosuppression. Nilotinib and dasatinib induced more early responses, and this suggested the possibility of an improved progression-free survival (PFS) and overall survival (OS) that may be significant. In practice, this expectation was not born out and there is now a considerable body of evidence suggesting that there is no additional benefit from achieving these molecular targets in terms of overall survival (OS) or PFS.[6-10] For this reason, no specific tyrosine kinase inhibitor (TKI) should be preferred over the others solely on the basis that it induces a higher proportion of molecular responses.[6] Are there any clinical factors that may influence the choice of first-line therapy? There is no doubt that the outcome of imatinib is worse in high-risk patients. These findings could justify the earlier use of nilotinib or dasatinib in these patients.[11,12] In conclusion, which is the best TKI? As recently stated, many think that "better" means "more effective".[16] If this is the case, there is no doubt that nilotinib and dasatinib are better. But if we consider other factors such as tolerability and/or toxicity, the situation may change and we should evaluate how many patients are still taking the drug at a given time point.[6] If a clinician would like to prescribe imatinib, he or she should do so only if the patient is evaluated for cytogenetic and early molecular response (3 months). Patients who do not achieve complete hematologic remission (CHR), CCyR and early molecular response should be switched to a 2nd generation TKI. Nilotinib and dasatinib both appear to be more effective than imatinib but this superiority must be confirmed over the next few years. The length of the observation time that shows the durability of the response and the lack of severe adverse events may count in favor of imatinib.

Minimal residual disease and discontinuation of therapy

Discontinuation of imatinib is currently an investigational therapeutic approach for patients with chronic phase (CP)-CML in prolonged complete molecular remission (CMR), defined here as a 4.5-log reduction in BCR-ABL/ABL levels and undetectable transcripts using reverse transcriptase quantitative polymerase chain reaction (RTQ-PCR). In 2007, the French team reported a pilot study to evaluate the feasibility and safety of imatinib discontinuation in patients in CMR for more than two years. They observed that half the patients experienced a molecular relapse during the six months following treatment discontinuation.[13] All patients in molecular relapse were re-treated with imatinib and regained CMR. No late molecular relapses were observed in the remaining patients with an extended follow up of more than

four years. These results were confirmed in the multicenter STop IMatinib (STIM) study.[14] The initial analysis reported the results of 69 patients with 12 months of follow up. Forty-two of them (61%) lost their CMR: 40 patients within the first six months, one patient at seven months, and another patient at 19 months. At 12 months, the probability of persisting CMR was 41% (95%CI: 29-52%). These results were confirmed in more patients and with longer follow up,[15] and also in three independent studies.[16-18]

Molecular relapse in the STIM study was defined as the finding of BCR-ABL transcripts in an assay with sensitivity of MR5 confirmed by a second successive analysis indicating an increase from the first analysis. However, during the molecular follow up of patients included in the pilot and STIM studies, investigators observed patients with fluctuating positivity of BCR-ABL transcript levels, suggesting that the definition of molecular relapse may need to be adjusted. They, therefore, evaluated whether the loss of MMR, defined as a 3-log reduction in BCR-ABL/ABL levels, is an accurate measure for defining molecular relapse after imatinib discontinuation. Next, they conducted a multicenter observational study "according to STIM" (A-STIM), initiated in June 2006, to evaluate the persistence of MMR in CML patients who had previously stopped imatinib after a prolonged CMR. Loss of MMR as a trigger for re-starting TKI therapy in CP-CML patients who stopped imatinib after durable undetectable disease identified a subset of patients for whom persistence of residual disease at a low level without any treatment does not necessarily mean the patient will proceed to cytogenetic or hematologic relapse. In very rare situations, they had previously observed this intriguing molecular pattern in patients treated with interferon-alpha. The key issue for the future will be to identify the factors responsible for the persistence of leukemic stem cells and even BCR-ABL signal positivity without an increase in the leukemic tumor burden in patients after treatment discontinuation.

The future of monitoring patients

In the future, new technology will be available to monitor patients with CML that could provide a more consistent interpretation of response between laboratories and could examine minimal residual disease to a greater depth. There is growing evidence to suggest that the initial molecular response is crucial for outcome and could determine therapeutic intervention.[19-21] The ability to detect minimal residual disease with high sensitivity is critical when determining eligibility for treatment discontinuation trials and to identify molecular recurrence.[14] Therefore, molecular monitoring of patients with CML should become a gold standard for monitoring patients and be readily available for all clinicians. However, despite efforts towards harmonization of molecular methods to measure BCR-ABL1 levels, there is still widespread inconsistency in results. The molecular methods are complex and require specialized skills and knowledge to ensure an environment free of contamination and consistency of the reported value. Resource limitations in many centers may preclude the establishment of valid molecular methods.

A relatively new technology, which may overcome the difficulties in developing a molecular method in individual laboratories, is an automated BCR-ABL1 assay that is contained within a single-use microfluidic cartridge. The platform uses a small volume of blood and incorporates RNA extraction, reverse transcription, quantitative PCR and result calculation.[22] A specialized instrument, the Cepheid GeneXpert, is required.[23] By incorporating conversion to the BCR-ABL1 international reporting scale, improvements in sensitivity and control of cartridge-to-cartridge variation this technique may become a powerful technological tool for monitoring patients with CML that can be readily incorporated into laboratory procedures.

An important approach to monitoring patients with TKI resistance is BCR-ABL1 kinase domain mutation analysis and the principal technique hitherto has been Sanger sequencing. However, it is now evident that more sensitive technology can provide important prognostic information. Mass spectrometry can improve the sensitivity of mutation detection by approximately 1-log compared with sequencing. A multiplex assay that incorporated 31 of the most frequently detected BCR-ABL1 mutations, which included the mutations known to confer a degree of clinical resistance to nilotinib or dasatinib, demonstrated that sensitive detection of resistant mutants can provide important prognostic information and aid therapeutic decisions after imatinib failure.[24,25] Patients with low-level mutations that were resistant to the TKI administered after imatinib failure had a poor outcome and rapid outgrowth of the low-level resistant mutants.[24] Furthermore, patients with more than one mutation detected by mass spectrometry and sequencing had a poor outcome, even if all of the mutations were predicted to be sensitive to the inhibitor received.[25]

In addition to the emerging relevance of low-level and multiple BCR-ABL1 mutations, certain compound mutations may be relevant for kinase inhibitor response or resistance.[26,27] These mutations occur in the same BCR-ABL1 molecule and cannot be distinguished using standard sequencing techniques. However, next-generation sequencing platforms can detect both low-level and compound mutations.[28] Due to limitations in the read lengths, most of these sequencers cannot differentiate compound mutations if they occur at a distance of more than 500 nucleotides. However, a single-molecule real-time (SMRT; Pacific Biosciences) sequencing platform can provide reads of sufficient length to interrogate the entire BCR-ABL1 kinase domain to distinguish polyclonal from compound mutations.[29] Interestingly, the initial studies using these technologies have determined that identical mutations may be acquired in parallel by separate cell populations in individual patients, and have revealed a complex combination of mutations. Further clinical follow up is required to assess the relevance of this complexity in terms of treatment response.

In summary, new technology for BCR-ABL1 quantification should enhance the efforts toward standardization of the reported value. New technologies for sensitive mutation detection could be important for determining the clinical consequence of clonal diversity of BCR-ABL1 and the co-existence of subclones.

Novel approaches for CML eradication

Whereas we know that TKI therapy is able to reduce the leukemic burden very substantially in most cases and almost eliminate it, the risk of disease progression is still the major cause of death. This is believed to be due to the persistence of Ph-positive progenitor cells including leukemic stem cells, which are more resistant to the TKI therapy than their more mature progeny.[30,31] However, more recently, at least two different sets of observations have introduced concepts that challenge the idea that life-long therapy with TKIs is mandatory in CML patients. First, the single-arm studies reported above suggest that discontinuation of imatinib can be successful in patients who achieved and maintained what the investigators define as CMR for a minimum period of at least two years.[14,16] Molecular recurrences are generally observed in approximately 60% of the cases within the first seven months after discontinuation and only occasional recurrences are observed at later time points. It is also worthy of note that few patients may exhibit a fluctuation in BCR-ABL transcript levels but without rising transcripts over time. Secondly, when nilotinib and dasatinib have been tested *versus* standard-dose imatinib as first-line therapy of CML, they not only showed increased capacity to induce a faster and higher rate of MMRs and to prevent some of the progression events that occur during the first months of imatinib therapy, but also the ability to substantially improve the percentage of patients achieving CMR, opening up the perspective of a possible definitive cure in a substantial number of patients.[32] Although we still do not know in which percentage of cases the treatment-free remission goal will be achieved by an intensive TKI therapy alone, this will certainly not be achieved in a reasonable time in those patients with a high number of leukemic progenitors and stem cells. Therefore, a better understanding of the mechanisms leading to CML leukemic stem cell survival in spite of the TKI therapy is needed to open up the future perspective of achieving CMR and, consequently, the possibility of reaching what has been defined as an "operational cure".[33]

This has led to an intense development of strategies aimed at targeting the stem cell signaling pathways that are involved in their maintenance and survival. Among several others, these pathways include the WNT–beta-catenin, Hedgehog, PML, SDF-1/CXCR4, BMP and Notch signaling pathways.[34] Autophagy has also been proposed as a potential target to eliminate leukemic stem cells.[35-37] Several strategies potentially useful to target these pathways have been proposed and include the use of small molecules, peptides and blocking antibodies. Importantly, however, inhibiting these signaling pathways can be toxic also for normal stem cells and further research is, therefore, needed to optimize their use in the clinical setting, as well as to explore potential combination therapies with other agents. Studies aimed at identifying the biomarkers that can be used to predict responses of individual patients to these treatments are also a priority for these potential new therapies. Also, despite these challenges, targeting the stem cell signaling pathways in CML patients is an attractive option to enable most of these patients to achieve treatment-free remission status in the near future.

From an immunological point of view, leukemia cells are nearly identical to their normal counterparts and this is the main reason why the host immune system allows them to grow in the body. However, the identification of leukemia-associated antigens (LAAs), such as proteinase 3 (PR3) and Wilms tumor antigen 1 (WT1), led to the development of peptide vaccines for myeloid leukemia.[38-40] However, although feasible, LAAs-based vaccination to treat residual Ph-positive leukemia remains a challenging task. Recent efforts have focused also on the development of adoptive immunotherapy of leukemia that is based on the administration of antibodies specific for leukemia antigens.

Recent therapeutic strategies to overcome resistance

Although most patients with CP-CML achieve long-lasting and profound responses to TKIs, targeting BCR-ABL1 resistance remains a significant clinical challenge, particularly in patients in accelerated or blastic phase (AP/BP). In newly diagnosed CP-CML patients, the 2nd generation TKIs, nilotinib and dasatinib, induce more rapid and more profound cytogenetic and molecular responses compared to imatinib, but refractory or relapsed disease still occurs in a subset of patients. Reactivation of BCR-ABL1 by mutations in the kinase domain (KD) of BCR-ABL1 is a well-defined mechanism of TKI resistance, but it has become clear that KD mutations fail to explain many cases of resistance.[41] In addition, the correlation between the *in vitro* sensitivity of KD mutants and the clinical response is close only toward the negative side, where the multi-resistant T315I mutation confers resistance to 1st and 2nd generation TKIs. On the other hand, many patients with supposedly 'sensitive' mutants are clinically resistant to 2nd generation TKIs.[42,43] Ponatinib, the first 3rd generation TKI to be approved, has demonstrated significant activity in heavily pre-treated patients with and without KD mutations.[44,45] This compound was rationally designed to avoid a requirement for a hydrogen bond with T315, skirting this residue in the distance.[27] In pre-clinical tests, ponatinib inhibited the proliferation of cell lines expressing a broad range of single BCR-ABL1 mutants, including T315I. Results from a phase I and a subsequent large phase II study (PACE trial) revealed high rates of MCyR and MMR.[46] In the latest update of the PACE trial, 54% of patients with CP-CML achieved MCyR (70% of those with T315I and 49% of those without T315). Fifty-eight percent of patients with AP-CML and 34% of patients with BP-CML or Ph-positive acute lymphoblastic leukemia achieved a major hematologic response. Most responses in CP-CML were stable, while relapse was common in patients with BP-CML. Preliminary data suggest that some cases of ponatinib resistance are associated with compound mutations of BCR-ABL1, i.e. mutations in the same allele, as predicted from *in vitro* mutagenesis assays.[47] Other patients developed resistance despite continued inhibition of BCR-ABL1, consistent with activation of alternative survival pathways, such as JAK/STAT. Whether this will emerge as a dominant resistance mechanism in patients who failed advanced BCR-ABL1 TKIs remains to be determined. Small molecule inhibitor and RNAi-based

assays have been developed to screen for pathways that confer BCR-ABL1 independent TKI resistance in cell lines and primary patient cells. Preliminary data suggest a crucial role for STAT3 in BCR-ABL independent resistance. In collaboration with Dr. Patrick Gunning (University of Toronto), Michael Deininger's team has optimized small molecules of STAT3 that block dimerization and nuclear translocation and are synergistic in combination with ponatinib against resistant CML cells, suggesting that BCR-ABL1 independent resistance can be overcome through synthetic lethality. Additional salvage pathways were identified through a lentiviral shRNA library screen and implicate nuclear-cytoplasmic transport and DNA repair in BCR-ABL-independent resistance. Given the previously reported role of STAT3 in extrinsically mediated TKI resistance,[48,49] they speculate that the mechanisms responsible for BCR-ABL1 independent TKI in patients with advanced CML may overlap with those responsible for the survival of CML stem cells in patients with minimal residual leukemia, despite continued TKI therapy. In this frame of thinking, extrinsic survival pathways activated by factors derived from the bone marrow microenvironment are responsible for the survival of residual CML stem cells, while activation of the same pathways by intrinsic mechanisms leads to relapse of active disease.

In conclusion, there is still much work to be done. In the near future, patients who are unfortunate enough not to have the complete benefit of the present TKI therapy could be managed with new drugs based on principles derived from the pioneering work in CML research.[50]

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Financial and other disclosures provided by the author using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are available with the full text of this paper at www.haematologica.org.

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