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NEOPLASIA

Comment on Chu et al, page 2093

Can we afford to let sleeping dogs lie?

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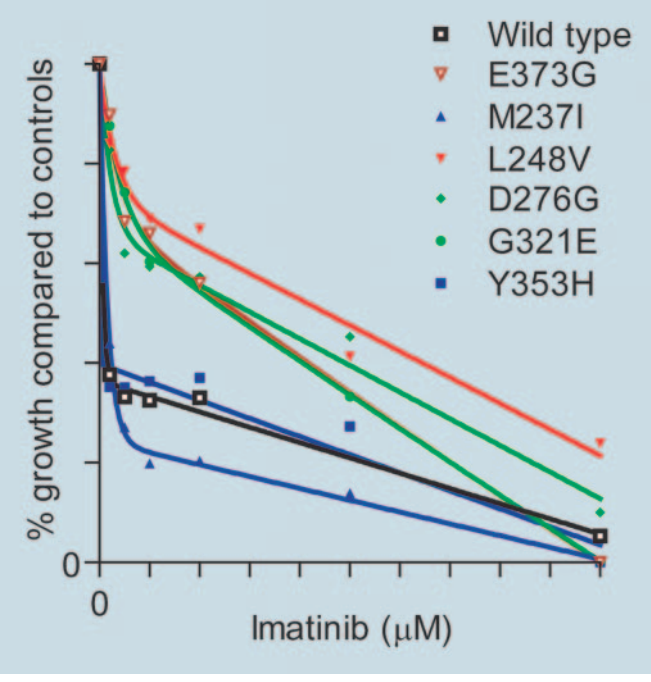
In patients with chronic myelogenous leukemia (CML) mutations of the BCR-ABL kinase domain (KD) have been identified as the leading cause of acquired resistance to imatinib, while the mechanisms underlying the persistence of minimal residual disease (MRD) are unknown. In this issue of *Blood,* Chu and colleagues report several patients with KD mutations at the time of complete cytogenetic response (CCR), implicating mutations as a cause of disease persistence.

Imatinib induces complete cytogenetic response (CCR) in most patients with chronic myelogenous leukemia (CML), but minimal residual disease (MRD) remains detectable by reverse transcriptase–polymerase chain reaction (RT-PCR) in all but a few cases. This is not just a cosmetic problem, as anecdotal observations reported rapid disease recurrence after discontinuation of imatinib, an indication that the residual BCR-ABL–positive cells retain full leukemogenic potential.[1,2] Furthermore, patients who receive imatinib as primary therapy for CML may progress to blast crisis directly from CCR.[3]



Sensitivity of TF-1 cells expressing KD mutants isolated from CML patients at the time of complete cytogenetic response to imatinib. See the complete figure in the article beginning on page 2093.

Reactivation of BCR-ABL kinase activity is common in patients who relapse after an initial response to imatinib. Most of these individuals harbor mutations in the kinase domain (KD) of BCR-ABL that impair drug binding.[4] In contrast, the mechanisms responsible for persistence of MRD in responding patients are not well understood. Chu and colleagues have studied $CD34^+$ cells from patients with CCR for KD mutations. They found mutations in 5 of 13 patients at the initial evaluation, and in 4 additional patients at follow-up, when rising BCR-ABL mRNA levels were detected by quantitative RT-PCR, while CCR was still maintained. Intriguingly, most of the mutations in these patients conferred only moderate resistance to imatinib in proliferation and phosphorylation assays, suggesting they may be capable of preventing the extinction of the leukemic clone but are barely able to support its expansion. Thus, KD mutations may be responsible for disease persistence in a subset of patients with CCR. A few issues, however, are curious. Although the frequency of mutations has not been studied systematically in patients with CCR, the incidence reported by Chu et al appears to be high compared with an unselected cohort of CML patients on imatinib.[5] One explanation for this discrepancy may be that the group under study may be high risk, consistent with the fact that the rate of overt relapse or rising levels of BCR-ABL mRNA on follow-up was certainly higher than one would expect in standard risk patients with CCR. Thus, these patients may have been caught on their path to disease progression rather than in stable remission. The other possibility is that the technique used—amplification of *Bcr-Abl* from $CD34^+$ cells and sequencing of multiple individual clones—may be instrumental for detecting mutant clones in CCR patients. Whether this approach would detect KD mutations at an appreciable frequency in patients with stable MRD must be addressed in future studies. Another intriguing observation is that mutants such as Y353H are equally or even more sensitive to imatinib than wild-type BCR-ABL but nonetheless grow out over time, suggesting that they may increase the transforming potency of BCR-ABL irrespective of imatinib or that another resistance mechanism may be present. From a therapeutic standpoint, it would be good news if KD mutants were found to cause disease persistence since they would be targets for alternative Abl kinase inhibitors.

Which other mechanisms may underlie disease persistence? Quiescent BCR-ABL–positive progenitor cells are present in CML patients that are capable of repopulating severe combined immunodeficient (SCID) mice. Treated with imatinib ex vivo, these cells survive drug concentrations that are lethal to proliferating CML progenitor cells.[6] There is

evidence that imatinib fails to significantly reduce BCR-ABL kinase activity in these cells at clinically achievable concentrations, although the precise mechanism for this remains elusive.[7] Possibilities include transporter proteins that affect intracellular drug concentrations such as OCT-1, P-glycoprotein, and ABCG2 or high levels of kinase active BCR-ABL protein. In this case, more potent inhibitors that are not substrates for these transporters should be able to eliminate MRD. However, yet another scenario is conceivable. CML stem cells may express BCR-ABL but they may not depend on it, relying on exogenous growth and survival signals, such as cytokines and interactions with stroma. Elimination of such dormant cells would require stem cell–directed rather than BCR-ABL–specific approaches. Whatever the precise mechanism of disease persistence, elimination of residual disease may be central to the long-term success of imatinib therapy of CML. ■

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CLINICAL OBSERVATIONS

Comment on Straathof et al, page 1898

"T"-ing off on nasopharynx cancer

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Nasopharyngeal carcinoma (NPC) is the most frequent Epstein-Barr virus (EBV)–associated malignancy worldwide. In this issue, Straathof and colleagues take aim at EBV-associated NPC showing that adoptive immunotherapy with EBV-specific T cells expanded in vitro can be successfully administered to NPC patients and is associated with potential therapeutic efficacy.

Epstein-Barr virus (EBV) immunotherapy has proved its mettle in stem cell transplantation where donor T cells are stimulated with autologous EBV-immortalized B cells in vitro and then transferred into the patient to prevent or treat uncontrolled proliferation of EBV-infected B cells, or so-called posttransplantation lymphoproliferative syndrome (PTLD). In these cases, EBV gene products expressed in the malignant B cell are ideal tumor-associated antigens marking tumor cells for killing by EBV-specific cytotoxic T cells (CTLs). It is not known which cell populations in the polyclonal T-cell mixture are responsible for therapeutic efficacy, but $CD8^+$ CTLs specific for the latent infection viral antigens expressed in immortalized B cells are presumed to be important.

Successfully applying this therapeutic approach to EBV-associated NPC is not without potential issues. The most obvious problem is a significant mismatch between the repertoire of $CD8^+$ CTLs and the pattern of EBV gene expression in NPC. EBV-immortalized B cells express 9 latent infection viral proteins—6 nuclear proteins (EBV nuclear antigens; EBNAs) and 3 latent membrane proteins (LMPs)—and CTLs stimulated in vitro by EBV-infected B cells most frequently recognize EBNA-3A, -3B, or -3C. However, NPC cells usually express only EBNA-1, LMP1, and LMP2, so these tumor cells fail to express the EBNA-3 targets recognized by most of the therapeutic CTLs. LMP1 is expressed in only 65% of the tumor cells and is not a very immunogenic protein, and LMP1-specific CTLs are uncommon. LMP2A protein is detected in only 50% of tumor cells, but LMP2A-specific T cells are a subdominant population following the EBNA-3s. EBNA-1 is important for maintenance of the viral episome, protein can be detected in every tumor cell, and it would be an ideal tumor marker. However, the EBNA-1 glycine-alanine repeat region has a potential immune evasion mechanism that prevents proteosome-dependent degradation and efficient presentation of EBNA-1 peptides through the HLA class I pathway, so that EBNA-1–specific CTLs fail to efficiently kill EBV-infected B cells in tissue culture.

Effective therapy also requires that the therapeutic T cells migrate to the tumor. The mucosal location and infiltrating T-cell milieu typically associated with NPC may provide additional obstacles for transferred effector T cells. NPC patients also present different logistic issues versus transplant patients. T cells from transplant donors can be prepared ahead of time, but this head start is not available for NPC patients.

The results reported by Straathof and colleagues show that these logistic problems can be overcome, and EBV-specific T-cell populations can be successfully generated for treatment of NPC patients. Cells were safely administered, and there was a trend for improved outcomes among the treated patients. These results are extremely encouraging since the protocol involved no significant alteration from the transplant patients. As expected, EBNA-3s were the dominant targets of the transferred T cells so that the bulk of the T cells may not have been expected to target the NPC tumor cells. The LMP2A-specific T cells represented less than 10% of the transferred T cells, so that specific manipulations to increase LMP2A-specific T cells may increase efficacy. The number of EBV-specific or LMP2A-specific T cells in the peripheral blood of treated patients did not significantly change, suggesting that a "full" lymphocyte pool may inhibit the in vivo amplification of the transferred EBV-specific T cells. The authors suggest that gentle immunodepletion prior to adoptive transfer may result in longer duration and efficacy of the EBV-specific T cells. An intriguing correlation was the best clinical response in a patient with a strong EBNA-1–specific response in the T-cell preparation. Recent studies indicate that EBNA-1–specific CTLs may have more effector activity