further reduction of BCR-ABL levels and without BM blastosis. The patient succumbed to CNS disease 3 months afterward, despite CNS-directed chemotherapy. Sequencing of the BCR-ABL kinase domain from BM blasts, at the time of BC, and CSF tumor cells, at CNS relapse, did not show any mutation. The lack of BM blastosis, the reduced marrow BCR-ABL levels and the absence of a mutated clone, in BM and CSF, at the onset of meningeal leukemia, indicate that systemic dasatinib failed to prevent CSF disease while still controlling extra-CNS leukemia. Thus, relapse was most probably due to suboptimal penetrance/activity of the drug in CNS given the low dasatinib dose (100 mg per day) used being the patient in CP. In the 4 months preceding CNS progression, the patient was not given comedications known to decrease the plasma concentrations of dasatinib,[4,5] including CYP4503A4 inducers, but rather received the CYP4503A4 inhibitor itraconazole. Therefore, even though we did not assess plasma levels, it is reasonable to exclude a negative influence of comedications on dasatinib bioavailability.

Dasatinib achieves CSF concentrations of 5.0% to 28% of those found in the plasma and Authors speculated that the biologic potency of dasatinib and/or the lack of protein binding may explain antitumor activity at low CSF levels.[1] However, approximately 50% of patients received steroids and/or other intrathecal agents before, together with, or soon after dasatinib, suggesting that this agent alone is unable exert a proper control of CNS disease in all cases.[1,6] Factors other than tumor cell resistance might influence dasatinib activity in the CNS, including suboptimal systemic dosing. Further pharmacokinetic studies are needed to identify patients in whom dasatinib alone may effectively control CNS disease.

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To the editor:

Does the presence of anti-HIV miRNAs in monocytes explain their resistance to HIV-1 infection?

We read with interest the article by Wang et al[1] that concluded that the presence of higher levels of anti–HIV-1 microRNAs (miRNAs) in monocytes may be responsible for their relative resistance to HIV-1 infection compared with macrophages.

They rightly point out that differential expression of the CCR5 coreceptor has been postulated as a reason for the differential susceptibility of monocytes and macrophages to HIV-1 infection. In our experience, only a small subset of monocytes ($< 5\%$) express CCR5 and this is at low level, whereas CD4 expression is also uniformly low compared with $CD4^+$ T cells. It is unclear from the experiments presented whether the CCR5 tropic viruses tested bind to monocytes or are internalized. A defect in adhesion or internalization would occur prior to any opportunity for miRNA to directly affect viral replication. Figure 1A from Wang et al[1] clearly demonstrates the negligible reverse transcriptase (RT) activity detected in supernatants of monocyte cultures after viral inoculation. However, it is unclear from the data presented the stage of the viral life cycle at which inhibition occurs. Therefore, despite the higher levels of anti-HIV miRNAs in monocytes, it has not been demonstrated that low-level expression of both CCR5 and CD4 is not the more significant factor in explaining their relative resistance to infection.[2]

Furthermore, in preliminary work from our laboratory, we checked miRNA expression in $CD4^+$ T cells from 8 healthy, uninfected controls and from 7 patients with chronic HIV-1 infection using a microarray system. Analysis of expression levels of the 4 miRNAs examined by Wang and colleagues showed that healthy control $CD4^+$ T cells expressed significantly higher levels of miR-28-5p and miR-150 (Figure 1), whereas miR-223 and

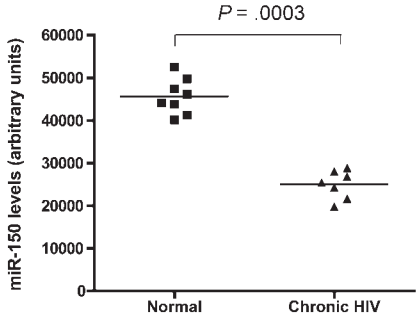

Figure 1. miR-150 levels in $CD4^+$ T cells. Shown are levels from 8 healthy volunteers (■) and 7 patients with chronic HIV-1 infection (▲) demonstrating a highly significant difference between the 2 groups. *P* value shown at top is by Mann-Whitney comparison of the 2 test groups.

miR-382 levels were not different between the 2 groups. It therefore appears that there is infection-related down-regulation of miR-150 and miR-28-5p in $CD4^+$ T cells during chronic HIV-1 infection. Using the same argument as the authors, we could conclude that because the uninfected controls had higher levels of miR-150 and miR-28-5p compared with the HIV-1–infected patients, $CD4^+$ T cells from healthy controls are relatively protected against HIV-1 infection. This conclusion is clearly incorrect with regard to $CD4^+$ T cells.

Our other concern is with the use of combined miRNA inhibitors to demonstrate changes in RT activity. Treatment of HIV-1–infected monocytes with miRNAs resulted in a rise in RT, but the effects were modest in comparison to RT levels achieved in HIV-1 infection of macrophages. It has been recently demonstrated that even inhibition of a single miRNA may modulate protein synthesis of thousands of genes.[3] The use of multiple inhibitors combined could lead to many unanticipated off-target effects. These may have unforeseen indirect effects on viral replication through changes in cellular phenotype or function. We contend that further studies confirming a lack of alteration in phenotype of monocytes after the use of these inhibitors is required before concluding that the change is a direct result of the actions of miRNAs on HIV protein synthesis.

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Response:

Intracellular restriction factors contribute to susceptibility of monocytes/macrophages to HIV-1 infection

Swaminathan et al[1] stated that despite the higher levels of anti-HIV microRNAs (miRNAs) in monocytes, there is no demonstration in our paper that the low-level expression of both HIV-1 primary receptor CD4 and coreceptor CCR5 on monocytes is not the more significant factor contributing to monocytes' resistance to infection. They argued that in their experience only a small subset of monocytes ($< 5\%$) express CCR5. This argument, however, has its limitation, given the fact that there is tremendous variability in CCR5 expression on primary monocytes from different donors. Several studies have shown that monocytes express relative high levels of CCR5.[2,3] We pointed out in the paper that although the demonstration of differential expression of CCR5 receptor on monocytes and macrophages provides an explanation for the differential susceptibility of these cells to HIV-1 infection,[4-6] this observation does not account fully for differences in HIV-1 infectivity in monocytes and macrophages. Peng et al showed there was no substantial difference in the expression of CCR5 in donor-matched monocyte and macrophage populations; in contrast, they found that CD4 and/or CCR5 expression decreased during macrophage maturation, despite increasing susceptibility to HIV-1.[7] They demonstrated that the expression of intracellur APOBEC3 is linked to susceptibility of monocytes and macrophages to HIV-1 infection.[7] Their funding in conjunction with ours strongly support the notion that intracellular restriction factors indeed contribute to susceptibility of monocytes/macrophages to HIV-1 infection.

Swaminathan et al compared their preliminary work using $CD4^+$ T cells with ours. However, there are significant differences in the study designs between theirs and ours. First, in our study we used monocytes and macrophages isolated from the same donors. In contrast, Swaminathan et al used $CD4^+$ T cells from the subjects of 2 different groups. Second, we examined the levels of the anti–HIV-1 miRNAs in donor-matched monocytes and macrophages before HIV-1 infection, whereas Swaminathan et al compared HIV-1–infected $CD4^+$ T cells with uninfected cells. As they suggested, it is possible that HIV-1 infection may down-regulate the expression of the anti–HIV-1 miRNAs in $CD4^+$ T cells, which explains the difference in the miRNA expression in $CD4^+$ T cells from the subjects of 2 groups. Third, in our study we were able to establish the association between the expression of the anti–HIV-1 miRNAs and HIV-1 infectivity in monocytes and macrophages, as we demonstrated that the suppression of the anti–HIV-1 miRNAs in monocytes facilitates HIV-1 infectivity, whereas increase of the miRNA expression in macrophages resulted in the inhibition of HIV-1 replication.

Swaminathan et al also raised the concern about the use of the miRNA inhibitors and their modest effect on HIV-1 reverse transcription (RT) in monocytes. The difference in modulation of HIV-1 infectivity in monocytes and macrophages transfected with the miRNA inhibitors or the miRNAs could be due to the fact that the transfection efficiency differed in monocytes and macrophages. We agree with the suggestion that future studies are necessary to determine whether the miRNA inhibitors have indirect effects on HIV-1 replication through changes in function and phenotype of monocytes and macrophages. Nevertheless, our demonstration that the cellular miRNAs that have anti–HIV-1 ability in $CD4^+$ T cells[8] are also associated with the protection of monocytes and macrophages from HIV-1 infection not only provides an additional explanation to address the question that has puzzled us for almost 2 decades but also offers insight into development of intracellular innate immunity-mediated therapeutics for HIV-1 infection and AIDS.