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Response:

Cybrd1 is not essential in mice

We appreciate the interest that Frazer and colleagues have shown in our work. However, in contrast to their interpretation, the purpose of our initial paper[1] was not to define the possible role of Cybrd1 (duodenal cytochrome b [Dcytb]) in intestinal iron absorption but rather to determine whether it is essential for the procurement of iron for utilization and storage in vivo. Our results clearly showed that it is not essential in129S6/SvEvTac mice, whether they were fed a standard lab diet or an iron-deficient diet.

Frazer and his coworkers are correct that our standard chow contains ferrous iron, possibly eliminating the need for an enzymatic ferric reductase. However, as we reported,[1] animals lacking Cybrd1 maintained iron stores comparable to wild-type mice after 8 weeks on an iron-deficient diet. In unpublished studies, we continued *Cybrd1*$^{-/-}$ mice on the iron-deficient diet for 6 months. Even under these conditions, their tissue iron stores and hematologic parameters were indistinguishable from wild type. Clearly, Cybrd1 is not essential for viability, for erythroid iron assimilation, or for maintenance of liver iron stores. While our data do not rule out a defect in intestinal iron absorption in *Cybrd1*$^{-/-}$ mice, they argue against a major role for Cybrd1 in vivo. For comparison, mice lacking the iron transporter Slc11a2 (divalent metal transporter 1 [DMT1]) in the intestine show a very severe reduction in liver iron stores and profound anemia that is undoubtedly due to a failure of intestinal iron absorption.[2]

We have not yet attempted to address whether Cybrd1 facilitates intestinal iron absorption. Other ferric reductases have been identified recently,[3] raising the possibility that there may be functional redundancy in dietary iron reduction. Future experiments should answer these questions.

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To the editor:

Activating *FLT3* mutations in CD4$^+$/CD8$^-$ pediatric T-cell acute lymphoblastic leukemias

Activating mutations in the FMS-like tyrosine kinase 3 gene (*FLT3*), including internal tandem duplications (ITDs) in the juxtamembrane (JM) domain or point mutations (PMs) in the activation loop, are the most common genetic aberration in acute myeloid leukemia (AML).[1] Recently, Paietta et al[2] investigated the presence of *FLT3* mutations in 69 adult T-cell acute lymphoblastic leukemia (T-ALL) patients. Three positive cases (2 ITDs and 1 PM) were identified sharing a similar early prothymocytic T-cell developmental state exclusively expressing cKIT/CD117, and a trial to test the efficacy of FLT3 inhibitors for this T-ALL subset was suggested.

To validate the incidence of *FLT3* mutations and to investigate a relation to outcome and other parameters, we screened 72 diagnostic pediatric T-ALL samples for *FLT3* mutations, as previously described.[3,4] We identified *FLT3*/ITD mutations in 2 pediatric T-ALLs (Figure 1A), whereas no point mutations in the kinase domain were detected. Sequence analysis confirmed a 51–base pair insertion in patient 2112 and a 57–base pair insertion in patient 1179 (Figure 1B). Moreover, no wild-type *FLT3* was identified in patient 2112, suggesting loss of the wild-type allele.[1]

Immunophenotypic analyses revealed a similar profile for both *FLT3*-mutated patient samples (ie, TdT$^+$, CD2$^+$, CD5$^+$, CD7$^+$, CD4$^+$/CD8$^-$, cytoplasmic CD3$^+$, surface CD3$^-$, and CD10$^-$). CD34 expression was detected in 24% and 21% of the leukemic blasts in patients 2112 and 1179, respectively. Only patient 2112 weakly expressed CD13 (24%) but not CD33. Although representing early T-cell differentiation stages for both patient samples, the maturation stage seems more advanced compared with the *FLT3*-mutated adult T-ALL cases (CD34$^+$, CD4$^-$/CD8$^-$).[2] Since no additional patient material was left for flow cytometry, *cKIT/CD117* expression was determined by real-time quantitative polymerase chain reaction (RQ-PCR) on isolated blasts[5] ($>$ 90% leukemic cells) from all pediatric T-ALL samples (Figure 1D). Whereas only the 3 *FLT3*-mutated adult T-ALL patients highly expressed cKIT,[2] most pediatric T-ALL samples expressed *cKIT* mRNA to some extent. Patient 2112 highly expressed *cKIT*, whereas patient 1179 showed a weak *cKIT* expression that was about 26-fold lower. Since various non–*FLT3*-mutated T-ALL samples highly expressed *cKIT/CD117* at levels comparable to patient 2112, we conclude that *cKIT/CD117* expression is not exclusively associated with *FLT3* mutations. Nevertheless, transcript levels do not necessarily correlate with protein expression levels.[2] In line with previous observations,[2] leukemic blasts of *FLT3*-mutated samples highly expressed *LYL1* and *LMO2*. Both pediatric samples carried a *HOX11L2* translocation in contrast to the *FLT3*-mutated adult T-ALL cases.[2]

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Figure 1. Activating FLT3 mutations in pediatric T-ALL. (A) Genomic PCR analysis for the *FLT3* gene. PCR results for 6 pediatric T-ALL patient samples are shown. T-ALL patient 1179 shows a heterozygous *FLT3*/ITD mutation. No wild-type *FLT3* is detected in T-ALL patient 2112, probably due to loss of heterozygosity of the wild-type allele. (B) Overview of the functional domains in the FLT3 tyrosine kinase receptor. Genomic position of the *FLT3*/ITD mutations detected in patients 1179 and 2112 are shown. Mutation position annotation is based on the *FLT3* reference sequence NM_004119. (C) *FLT3* mutation analysis of diagnosis and relapse material of T-ALL patient 1179. The *FLT3*/ITD mutation was present at diagnosis but absent at relapse. (D) Relative *cKIT/CD117* mRNA expression indicated as percentage of *GAPDH* expression for the investigated pediatric T-ALL cohort (*FLT3* wt vs *FLT3* mut). For both groups the median *FLT3* mRNA expression is shown. wt indicates wild type; mut, mutated; dx, at diagnosis; R, at relapse; and *, genomic position of the recently identified *FLT3*/ITD mutations in adult T-ALLs.[2]

Patient 1179 relapsed 13 months after initial diagnosis, whereas patient 2112 is in continued complete remission (CCR; 61^{+}). Interestingly, patient 1179 showed no *FLT3*/ITD mutation at relapse (Figure 1C), possibly due to loss of the mutated allele during therapy, or, alternatively, the *FLT3*/ITD-positive clone was eliminated during chemotherapy with a subsequent relapse from a non–*FLT3*-mutated parental clone.

In conclusion, we confirm the presence of *FLT3* mutations in pediatric T-ALL (2/72; 2.7%). Although both immature, the immunophenotypes of the *FLT3*-mutated pediatric and adult[2] T-ALL cases differed. In addition, a link between mRNA expression of *cKIT/CD117* and *FLT3* mutations could not be demonstrated. Since patient 2112 is in CCR and relapse material of patient 1179 did not show evidence for *FLT3* mutation, the *FLT3*-mutated T-ALL subclone seems to be effectively eradicated by current chemotherapy. This suggests that the application of FLT3 inhibitors for *FLT3*-mutated T-ALL, as suggested by Paietta et al,[2] may not further improve treatment outcome in pediatric T-ALL.

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To the editor:

Sickle cell disease and hyperreactive malarial splenomegaly (HMS) in young immigrants from Africa

Hyperreactive malarial splenomegaly (HMS) is the most frequent cause of massive tropical splenomegaly in areas with a stable transmission of malaria.[1] Studies on the pathogenesis of HMS suggest a critical role of aberrant immunologic response to malaria antigens after repeated infections, resulting in splenic hypertrophy, sometimes associated with secondary hypersplenism.[2-6] The prognosis of untreated HMS is unfavorable with mortality of 50% in endemic areas, up to 85% in the presence of severe splenomegaly.[1]

In malarious areas, the high frequency of hemoglobinopathies, such as sickle cell disease (SCD), support their protective role against *Plasmodium falciparum* malaria.[7-9] However, in patients homozygous for sickle hemoglobin (SS), the persistence of unrecognizable *P falciparum* infection could trigger acute hemolytic[4] events and/or recurrent vaso-occlusive crises (VOCs). Since splenomegaly is the clinical manifestation common to both diseases, HMS might be easily overlooked in SS patients in malaria-endemic countries or in SS subjects recently emigrated to Europe or North America from Africa.

Here, we describe two SCD patients recently emigrated from Africa, with large splenomegaly and hypersplenism, who were