

KIT/Val⁶⁵⁴Ala Receptor Detected in One Imatinib-Resistant GIST Patient

To the Editor: We read with interest the article entitled "A Missense Mutation in *KIT* Kinase Domain 1 Correlates with Imatinib Resistance in Gastrointestinal Stromal Tumors" by Chen et al. because we have recently found the same mutation in one of our surgically treated gastrointestinal stromal tumor patients. This patient had a metastatic gastrointestinal stromal tumor carrying a *KIT* exon 11 mutation (V559A) and underwent surgery because of clinical and radiological disease progression after imatinib treatment. Molecular analyses revealed an exon 11-activating mutation in all but one of the tumoral metastatic nodules analyzed, whose features were consistent with active proliferation and carried the adjunctive exon 13 mutation (T1982C) responsible for Val⁶⁵⁴Ala substitution. Biochemical analyses revealed a *KIT* receptor that was equally phosphorylated and expressed in all the specimens regardless of the genotype. The karyotype was normal in all nodules, in line with the findings of Chen et al.

However, we would like to comment on the unusually high frequency of the T1982C mutation in their case material (5 of 12 patients), which is strikingly different from the considerably lower percentage of point mutations in gastrointestinal stromal tumors with acquired resistance to imatinib observed by us. Among eight gastrointestinal stromal tumor patients progressing after imatinib treatment characterized by molecular/biochemical and cytogenetic analyses, we identified only two point mutations in two different patients, one responsible for T670I substitution (1) and the other identical to that detected by Chen et al.

Although their finding of an association between the mutation (with a normal karyotype) and disease progression under drug treatment is impressive, functional experiments attesting actual *KIT*/Val⁶⁵⁴Ala imatinib resistance and its possible kinase-activating effect are strongly recommended to support this assumption. It is only with transfected cells expressing the double *KIT* mutant (carrying exons 11 and 13 mutations on the same allele) and treated with different drug doses can provide useful information concerning the critical imatinib dose (if any) needed to deactivate/dephosphorylate the mutated receptor. As all their patients were treated with imatinib 400 mg/d, it is not known whether increasing the dose to 800 mg/d may prevent the development of resistant clones. We transfected COS1 cells with *KIT*/559/T670I and showed that this receptor is resistant to imatinib 15 mol/L, a clinically unachievable concentration.

To acquire further insights into imatinib resistance and test new inhibitory molecules, suitable problem-oriented *in vitro* experiments coupled with molecular modeling are required.

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Reference

1. Tamborini E, Bonadiman L, Greco A, et al. A new mutation in *KIT* receptor ATP pocket is responsible for acquired resistance to imatinib in a patient with gastrointestinal stromal tumor. *Gastroenterology* 2004;127:294-9.

In Response: We appreciate Dr. Tamborini's letter and their thorough and interesting single case report of T670I substitution in one imatinib-resistant gastrointestinal stromal tumor (GIST) patient. We are especially grateful to Dr. Tamborini for sharing with us their recent unpublished finding of the same 1982T→C mutation (Val654Ala) in one of their imatinib-resistant GIST, which further strengthens the *in vivo* correlation of imatinib resistance and this mutation reported in our study. We will first address the first comment on mutation frequency. Our reported 1982T→C mutation (Val654Ala) represents a very early and a very effective mutation associated with imatinib resistance as shown in our report. One possible reason of our high detection rate of this mutation is the stringent criteria of imatinib resistance and selection of implants (equivalent to *in vivo* clones) demonstrating true rapid unequivocal progression with estimated doubling time of ≤35 days as shown in Fig. 1 of our report. There is distinctive differences between the truly resistant GIST and the residual GIST. A second reason may be because these five imatinib-resistant GIST implants were surgically resected immediately after detection by computed tomography scans within 3 months of their emergence of imatinib resistance. Fortunately, imatinib resistance is infrequent and the identification of these five cases in our report spanned over 1 year while treating ~130 GIST patients (106 GIST patients in S00-33 clinical trial and >25 GIST patients treated off protocol). The true rate of Val654Ala mutation in imatinib-resistant GISTs is likely to vary until sufficient cases have been studied to obtain statistically significant estimate. In addition, more than half of our imatinib-resistant GIST patients are not surgical candidate, and biopsy is neither justified nor indicated in the S00-33 protocol, thus preclude molecular studies.

Our report presented five cases of convincing temporal and causal association of imatinib resistance and the novel mutation 1982 T→C (Val654Ala) *in vivo*. Further *in vitro* studies are in progress. We agree with Dr. Tamborini that transfection experiments using double *KIT* mutant will provide invaluable *in vitro* model to study imatinib resistance and develop new drugs.

All five GIST patients A to E are refractory to 800 mg/d imatinib. It remains unknown whether starting with 800 mg/d (instead of 400 mg/d) imatinib could have prevented or delayed imatinib resistance. The S00-33 clinical trial (Dr. George Demetri, principal investigator) and translational study results will provide more valuable information and statistics.

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