Molecular Heterogeneity of Kinase Inhibitor Resistance Mechanisms in Gastrointestinal Stromal Tumors

G. Ravegnini [1,2],S. Angelini [1],P. Hrelia [1], J. A. Fletcher [2]
[1]Dept. of Pharmacy and Biotechnology, University of Bologna, Bologna
[2]Dept. of Pathology, Brigham and Women's hospital and Harvard Medical School, Boston, MA, USA

Background: Clinical progression of metastatic gastrointestinal stromal tumor (GIST), during tyrosine kinase inhibitor (TKI) therapy, is often multifocal. However, TKI resistance mutations are assessed in only single, or few, progressing metastases (mets) per patient (pt). We used high-throughput screens to evaluate TKI resistance mechanisms in up to 40 progressing GIST mets per pt.
Methods: Clinically progressing *KIT*-mutant GISTs were from pts formerly responding to imatinib and/or sunitinib. *KIT* exons 8 through 18 were sequenced at 2000-fold coverage (454 pyrosequencing) and these analyses were confirmed and extended to additional mets from the same pts. Drug-response studies were performed by expressing mutant constructs in a KIT-negative GIST model.
Results: 454 *KIT* sequencing was performed in untreated GISTs (N=25), responding GISTs (N= 14) progressing GISTs (N= 37), and non-GIST sarcomas (N = 5). Secondary *KIT* mutations (in addition to the known primary mutation) were demonstrated in 3 untreated GISTs (13%), but were rare events (<4% of *KIT* sequences). Secondary *KIT* mutations were found in 33 progressing GIST mets (94%), of which 7 mets had 2 or more resistance mutations in the same <2mm^3 sample. analyses revealed a maximum of 7 different predominant secondary *KIT* mutations (each mutation found in >25% of *KIT* alleles from at least one met) among 40 geographically discrete progressing mets, from one patient. Novel sunitinib resistance mutations, in pts with *KIT* exon 9 primary mutation, involved *KIT* ex 11 (del-ins), ex 13-14 (N655S, N680K and F681L), and ex 18 (S840N). All *KIT* secondary resistance mutations were on the same allele (cis) as the primary mutation. Nilotinib and sorafenib inhibited a subset of these mutations but were ineffective against others.
Conclusions: Systematic genomic evaluations demonstrate up to 7 TKI resistance mutations per pt, in different progressing GIST mets. These complex molecular resistance mechanisms can be in part inhibited, *in vitro*, by novel therapeutic strategies