Exome Sequencing Informs Mechanisms of Clinical Resistance to the FLT3 Inhibitor Crenolanib

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Abstract

In conclusion, rately FLT3 mutations were present in AML cases, and a prominent feature of drug resistance in the setting of FLT3-ITD. Crenolanib treatment of AML patients with FLT3-ITD and/or FLT3-ITD clones exhibited a FLT3 gatekeeper mutation despite good coverage of the FLT3 locus (average of 134 fold read depth; 560 2602 223 324 1). We report that a minority of patients acquired FLT3 gatekeeper or other secondary FLT3 mutations in vitro. In addition, our in vitro drug resistance in the setting of FLT3-ITD. Crenolanib treatment of AML patients exhibiting FLT3 D835 and/or FLT3-ITD was reduced, but a drug resistant subclone expanded during treatment.

Introduction

To determine additional somatic mutations in the exome that may be acquired at the time of crenolanib resistance.

Methods

Patient population: Samples in this study were collected from relapsed/refractory AML, patients who received crenolanib as monotherapy. The sampled population included patients who had durable responses to crenolanib, as well as those who have not had responses.

Whole Exome Sequencing: To determine the potential mechanism of crenolanib resistance, the bone marrow and peripheral blood samples from 42 patients who had FLT3-ITD mutations with clones were assayed by whole exome sequencing. We performed whole exome sequencing using Illumina NextSeq 500 and paired-end sequencing on an Illumina HiSeq 2500. We performed standard quality control procedures before analysis to minimize false-negative results and minimize the impact of PCR bias in the context of clinical therapy. For the other 17 patients, samples at the time of failure were assayed.

Ultraprofiling: Exome 17 and exon 20 of FLT3 were also sequenced in 20 patient samples to analyze an average of 228 genes, including genes involved in cell survival, cancer development, and drug resistance.

Detected Three Novel Mutations of FLT3

Three (3) novel mutations were identified, and two (2) of these were present of FLT3 mutations. Among the 19 patients, 14, 19, and 29 (100%) were diagnosed with FLT3-ITD, FLT3-ITD and TKD, and FLT3-ITD, respectively. The presence of these mutations was confirmed by Sanger sequencing in a subset of samples.

Detected FLT3 Gatekeeper Mutation F891L Was Infrquent in Crenolanib-treated AML Patients

Patient 1496 developed a F691L mutation during crenolanib treatment (Figure 2). This mutation was observed in 34% of cases (50/150) and was also observed in the C2D1 line, where it was present in 7% of cases (48/660). In addition, the mutation was also observed in the C3D1 line, where it was present in 10% of cases (76/760). The presence of this mutation was confirmed by Sanger sequencing in a subset of samples.

Crenolanib Has Activity Against FLT3 A833S, D839Y/G and N841K A-loop Mutations

Patient 4592 responded to crenolanib and achieved CR after one cycle of crenolanib (28 days). Patient 4593 responded to crenolanib and achieved CRi after one cycle of crenolanib (28 days).

Detected Other FLT3 Mutations in In Vitro

We detected a FLT3 gatekeeper mutation (ITD + D835) in 5.3 ± 2.7 cases exhibiting a FLT3 gatekeeper mutation despite good coverage of the FLT3 locus (average of 134 fold read depth; 560 2602 223 324 1) before and after crenolanib treatment. We observed a prominent signal of acquired mutations in transcriptional regulatory mutations, suggesting a more biologically aggressive mechanism of resistance to crenolanib.