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Using CRISPR-induced RUNX1 mutations in the HMC-1.2 cell line to study systemic mastocytosis

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Abstract

Systemic mastocytosis (SM), is a rare blood disorder that is characterized by the over-proliferation of mast cells caused by a gain-of-function mutations in KIT, a receptor tyrosine kinase. The most common mutation in KIT is D816V, which causes ligand-independent activation of the receptor. Inhibitors of KIT-D816V can be highly effective in reducing mast cell proliferation, but some patients develop drug resistance. Drug resistance and poor prognosis of patients with SM is associated with mutations in additional genes such as RUNX1, a transcription factor important for the formation and maturation of blood cells. We hypothesize that RUNX1 mutations may confer resistance to drugs targeting mutant KIT. To test this hypothesis, we used the Lenti-X CRISPR/Cas9 system on a human systemic mastocytosis cell line (HMC-1.2) to introduce a RUNX1 truncating mutation commonly observed in patients with SM. These HMC-1.2 cells have a KIT-D816V mutation and are highly sensitive to the KIT inhibitor, avapritinib. Successful introduction of the RUNX1 mutation using CRISPR was measured via polymerase chain reaction (PCR) and Tracking of Indels by DEcomposition (TIDE) analysis, which revealed a 70% indel frequency indicating the editing efficiency. We treated RUNX1-mutant cells with avapritinib for 72-hours and assessed inhibition of cell growth using an MTS cell proliferation assay. We observed no statistically significant change in sensitivity of these cells to avapritinib relative to the controls. Although short term response to avapritinib was not affected, potentially long-term resistance may still occur and will require further evaluation.