Supplementary Methods

Droplet digital PCR for MET D1228V

We developed a MET D1228V ddPCR assays similarly as previously described. In brief, we custom designed primer and probe pairs, optimizing them for annealing temperature and cycling condition using serial dilutions of wild type MET and MET D1228V plasmid DNA. Forward primer, 5′-CAGTCAAGGTTGCTGATTTTGGT-3′, reverse primer, 5′-TGCACCTGTTTTGTTGTGTACACTA-3′. Probe sequences: 5′-VIC-CTTGCCAGAGACATGTAT-MGB-NFQ-3′, 5′-FAM-TTGCCAGAGTCATGTAT-MGB-NFQ-3′. All ddPCR reagents were purchased from Bio-Rad, and custom ordered primers and probes were from Life Technologies. ddPCR analysis was performed on a Bio-Rad QX100 ddPCR instrument with the following PCR conditions: Cycling conditions: 95 °C × 10 min (1 cycle), 40 cycles of 94 °C × 30 s and 57 °C × 1 min, and 10 °C hold.

Molecular docking study

Co-crystal structures of c-Met kinase domain in complex with PF-04254644 (type I kinase inhibitor) or foretinib (type II kinase inhibitor) were retrieved from Protein Data Bank (PDB code 3ZC5 and 3LQ8). The retrieved protein–ligand structures were processed by using Protein Preparation Wizard (1): addition of all hydrogens, assignment of bond orders, deletion of all water molecules, capping uncapped termini. Any missing side chains and missing loops were filled by using prime module (2) integrated within Protein Preparation Wizard. All residues of the proteins were then parameterized using OPLS2005 force field (3). Restrained minimization was performed until the converged average root mean square deviation (RMSD) of 0.3 Å was reached for heavy atoms. The c-Met mutant (D1228V) was generated by simple amino acid substitution and the geometry was optimized using of OPLS2005 force field. Docking studies of savolitinib and cabozantinib on c-Met kinase domain (wild-type and D1228V mutant) were carried out using

GLIDE module (4) in Schrodinger's package. A docking grid defining c-Met kinase domain was generated mainly considering the binding pocket of the c-Met inhibitors. Ligands (savolitinib and cabozantinib) were prepared using the Ligprep module (5) of the Schrodinger packages.

Molecular Dynamics Simulations

Molecular dynamics (MD) simulations of the complexes of c-Met kinase domain (wild-type and D1228V mutant) with these inhibitors (savolitinib and cabozantinib) were performed using the Desmond (6) in Schrodinger2015-4 software. Predefined TIP3P water model was utilized to simulate water molecules using OPLS2005 force field. Orthorhombic periodic boundary conditions were set up to specify the shape and size of the repeating unit buffered at 10 Å distances. In order to neutralize the system electrically, sodium ions were randomly placed in the solvated system to balance the system charge. After building the solvated system containing the protein in complex with the ligand, the system was minimized and relaxed using default protocol integrated within Desmond module using OPLS2005 force field parameters. Molecular dynamics simulations were carried out with the periodic boundary conditions in the NPT (isothermal and isobaric simulations) ensemble. Nose–Hoover thermostat algorithm (7) and Martina-Tobias-Klein method (8) were adopted for constant-temperature (300 K) and isotropic pressure (1 atm), respectively. The calculations were carried out by running the 500 ns NPT production simulation and saving the configurations. Trajectory was obtained at 500 ps intervals.

References

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