Mass Spectrometry analysis of AZD9291 covalent binding to EGFR(L858R/T790M)

Recombinant EGFR (T790M) was incubated with AZD9291 in the presence of human serum albumin. The untreated and treated sample were run onto an SDS-PAGE gel and stained with Coomassie Blue. The EGFR (T790M) bands were excised from the gel; they were then subjected to washes with 50mM ammonium bicarbonate and acetonitrile to dehydrate the gel pieces. The bands were incubated with 10mM dithiothreitol for 30 minutes at 65°C followed by a further incubation in 15mM iodoacetamide for 30 minutes at room temperature in the dark. The gel bands were then subjected to more washes with 50mM ammonium bicarbonate then dehydrated with acetonitrile. Chymotrypsin (Roche) in 50mM ammonium bicarbonate was added and the samples were left at room temperature to digest overnight. The supernatent from around the gel pieces was extracted and then further peptide extractions were carried out with 0.1% trifluoracetic acid/60% acetonitrile. The extracts were concentrated to remove the acetonitrile. Analysis was performed using a NanoAcquity UPLC system coupled to a Synapt G2 mass spectrometer (Waters). 4.5 µl of sample was injected per sample and the peptide mixture was fractioned using a 25 cm x 75 µm, 1.7 µm particle, C18 column using a gradient of 3% to 40% acetonitrile over 20 min. The Synapt G2 operated in MSe mode with a collision energy ramp of 15-35 eV in the high energy scan. Glu-fibrinopeptide was continuously infused as a lockmass for mass correction. The resultant raw data files were searched using Biopharmalynx software (Waters). A specific analysis was created containing the recombinant EGFR(T790M) sequence along with the mass adduct of 499.2696Da for the compound AZD9291.
Figure S1

A

Untreated EGFR(T790M) - 591.78 Da MH^2+ ion not identified

AZD9291 treated EGFR(T790M) - 591.78 Da MH^2+ ion identified

B

MH^+ mass of 773.38 Da b-ion detected equating to the fragment Gly-Cys-Leu with the AZD9291 compound mass addition.
Figure S1. AZD9291 covalently binds to cysteine-797 in EGFR(L858R/T790M).

A. Mass spectrometric analysis of recombinant EGFR(L858R/T790M) treated with AZD9291 in the presence of human serum albumin. Chymotryptic peptides derived from untreated and treated samples were analysed by Mass Spectrometry. Data shows expected increase in mass of Cys797 peptide, GCLLDY, following covalent modification by AZD9291. B. MSMS confirms the mass addition of the AZD9291 compound on the GCLLDY peptide with an MH$^+$ mass of 1182.58Da, the identified fragment ions are labelled.