

Additional file

Article title: Larotinib in Patients with Advanced and Previously Treated Esophageal Squamous Cell Carcinoma with Epidermal Growth Factor Receptor Overexpression or Amplification: An Open-Label, Multicenter Phase 1b Study

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Additional file 5: Preclinical Kinase Inhibition Assays

Purpose: To understand the difference in activity between larotinib and other similar drugs, we compared the inhibitory activity of larotinib, gefitinib, erlotinib and icotinib on EGFR family kinases in the same batch.

Methods: The inhibition profile of larotinib and other similar drugs against EGFR family kinases was determined using Z'-LYTE biochemical assay and the LanthaScreen Eu Kinase Binding Assay. To determine half-maximal inhibitory concentration (IC50) values, we set ATP concentrations at the Km for each kinase and measured phosphorylation of peptide substrate polymers.

Results: To assess the potential of larotinib to inhibit EGFR family kinases, and to compare its activity with that of the other EGFR inhibitors, we screened the compounds against 11 EGFR family kinases. The results showed that larotinib inhibited the kinase activity of wild-type EGFR and L858R-mutant EGFR significantly, and had potent binding affinity for del19-EGFR. The activities of gefitinib and erlotinib against EGFR-wt and EGFR-mut were similar to those of larotinib. However, larotinib had a stronger ability to inhibit EGFR kinase compared to icotinib (Table S4).

Table S4 *In vitro* inhibitory activity against EGFR-family kinases of larotinib and similar drugs

Kinase	IC ₅₀ (nM)			
	Larotinib	Gefitinib	Erlotinib	Icotinib
	Z650	Z490	Z621	Z773
EGFR (ErbB1) C797S	7.24	7.18	11.7	38.7
EGFR (ErbB1) G719C	3.54	8.64	3.29	13.1
EGFR (ErbB1) G719S	7.05	10.1	5.84	15.7
EGFR (ErbB1) L858R	0.254	0.452	0.467	2.03
EGFR (ErbB1) L861Q	0.298	0.369	0.269	1.25
EGFR (ErbB1) T790M C797S L858R	264	402	406	1390
EGFR (ErbB1) T790M L858R	101	168	231	1140
EGFR (ErbB1) T790M	40.9	161	368	1080
EGFR (ErbB1)	0.371	0.374	0.554	1.54
EGFR (ErbB1) d746-750	0.203	<0.150	0.248	1.04
EGFR (ErbB1) d747-749 A750P	0.330	0.277	0.342	1.30