YH25448, a highly selective 3rd generation EGFR TKI, exhibits superior survival benefit over osimertinib in mice with brain metastases of NSCLC

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BACKGROUND & OBJECTIVES

Currently-available EGFR tyrosine kinase inhibitors (TKIs) are ineffective for the treatment and prevention of brain metastases (BrM) due to the limited blood-brain barrier (BBB) penetration. YH25448 is a potent, highly mutant-selective and irreversible 3rd generation EGFR TKI that is able to penetrate the BBB, and targets both the T790M mutation and activating EGFR mutations while sparing wild type (wt). In vitro biochemical, functional experiments, in vivo PK and pharmacology/pharmacodynamic studies were performed to characterize the non-clinical profile of YH25448 using relevant assays, cell lines and animal models.

METHODS

[In Vitro Pharmacology]

◆ **Kinase assay**
- ✓ Cell-free kinase assays were conducted using time-resolved fluorescence resonance energy transfer (FRET™) technology. The ATP concentration corresponding to the Km of each enzyme was used. Kinase reactions were incubated at room temperature for 1 hour and the IC_{50} values were determined using GraphPad Prism™ software.

◆ **Anti-proliferation assay**
- ✓ Human NSCLC cell lines or patients-derived cells with various EGFR mutant status were incubated with test compounds. After 72 hours, cell viability was measured by quantifying the total amount of ATP using an ATPLite™ assay kit.

◆ **p-EGFR assay**
- ✓ To determine whether its cellular anti-tumor activity against NSCLC cells correlated with molecular potency as assessed by change in phosphorylation of EGFR mutant protein, YH25448 or the reference inhibitors were incubated for 2 hours in representative NSCLC cells, followed by Western blot analysis.

◆ **Apoptosis assay**
- ✓ To understand the mechanism responsible for the anti-cancer activity of YH25448, we investigated cell death signaling induced by EGFR inhibition. Since caspase-3/7 are the key regulators of apoptosis, caspase-3/7 activity was determined in H1975 and PC9 cells after 48 hr incubation with each inhibitor.

[In Vivo Efficacy and Pharmacokinetics/Pharmacodynamics]

◆ **H1975 subcutaneous implantation and brain metastases model**
- ✓ Luciferase-transfected H1975 human NSCLC cells were implanted into right flank and/or brain in female BALB/c nude mice.
- ✓ Tumor burden of intracranial lesions and tumor size of right flank were measured using a bioluminescence imaging (BLI) technique and a digital caliper, respectively.
- ✓ Established tumor bearing mice were treated with once daily oral dose of YH25448 or AZD9291 from 13 days post implantation (n=7-8/group).

◆ **In vivo pharmacodynamic study**
- ✓ In H1975 human NSCLC subcutaneous xenograft model, tumor tissue were collected at 2hr, 8hr and 24hr after 14 days treatment and p-EGFR and total EGFR were evaluated by Western blotting.

◆ **Pharmacokinetics**
- ✓ Blood, tumor, brain, or CSF samples were collected from the animals at the designated time points and concentration of YH25448 was determined using a validated LC-MS/MS method.

◆ **Statistics**
- ✓ All data are presented as mean ± SEM. Data were analyzed by using one-way ANOVA followed by Dunnett's test or Student's t-test. The survival analysis was performed using Kaplan-Meier survival curve and log-rank test comparing each group. *:p<0.05, **:p<0.01, ***:p<0.001 vs. vehicle, #:p<0.05, ##:p<0.01, ###:p<0.001 vs. AZD9291

RESULTS

YH25448 is a potent and highly mutant selective EGFR inhibitor and it induces apoptosis of NSCLC cells through induction of Bim

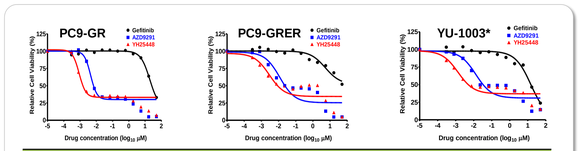
Fig. 1. In vitro kinase inhibition activity

Kinase	IC50, nM	
	AZD9291	YH25448
EGFR (L858R/T790M)	8	2
EGFR (T790M)	2.2	1.7
EGFR (L858R)	12.2	20.6
EGFR (del19)	8.6	5.3
ErbB2	44	364
ErbB4	54	1,017
Wild type EGFR	54	76
IGF1R	1,023	>3,000

Fig. 2-1. In vitro anti-cancer activity

Cell lines	EGFR genotype	GI_{50}, nM		
		Afatinib	AZD9291	YH25448
H2073 (+EGF)	Wild type	6.4	244.4	318.1
PC9	del19	2.3	4.8	2.7
H1975	L858R/T790M	6.9	11.8	3.6

Fig. 2-2. In vitro anti-cancer activity of YH25448



Cell lines	EGFR genotype	GI_{50}, nM		
		Gefitinib	AZD9291	YH25448
PC9	del19	70.6	15.3	2.2
PC9-GR	del19+T790M by Gefitinib	> 15000.0	4.5	0.9
PC9-GRER	del19+T790M by Gefitinib & EGF816	5321.0	11.9	2.9
HCC827	del19	53.4	1.8	0.7
H1975	T790M+L858R	> 15000.0	18.7	6.3
YU-1003*	del19	> 15000.0	13.1	1.3
YU-1005*	Wild type	13162.0	4011.1	5128.5
H1666	Wild type	1838.0	333.9	377.5
H2291	Wild type	> 15000.0	4345.0	5506.0

* NSCLC patient-derived cell line

Fig. 3. Apoptosis inducing activity

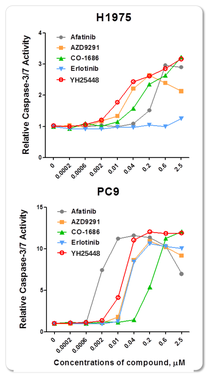
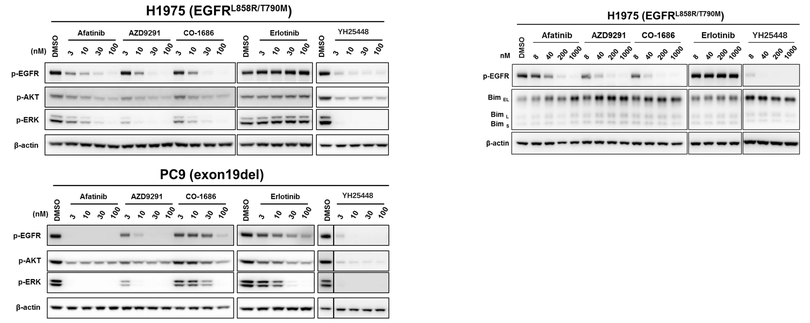


Fig. 4. Down-regulation of p-EGFR and down-stream p-AKT and p-ERK signaling by YH25448 leads to induction of Bim in EGFR mutant NSCLC cells



YH25448 showed excellent efficacy in a brain metastases model with NSCLC cells carrying the T790M mutation, and provided superior survival benefit over AZD9291 with good tolerability

Fig. 5. Dose response efficacy of YH25448 in H1975 subcutaneous and intracranial dual implantation tumor model in BALB/c nude mice

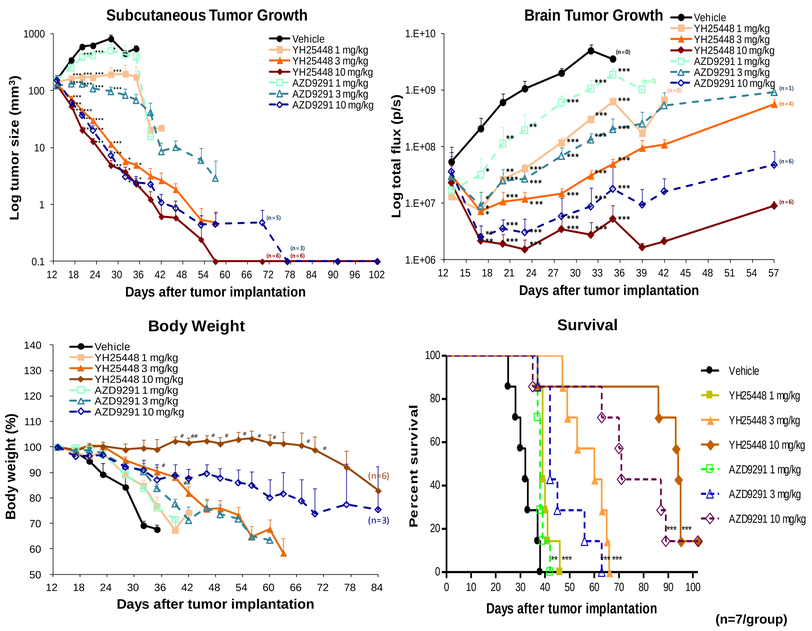
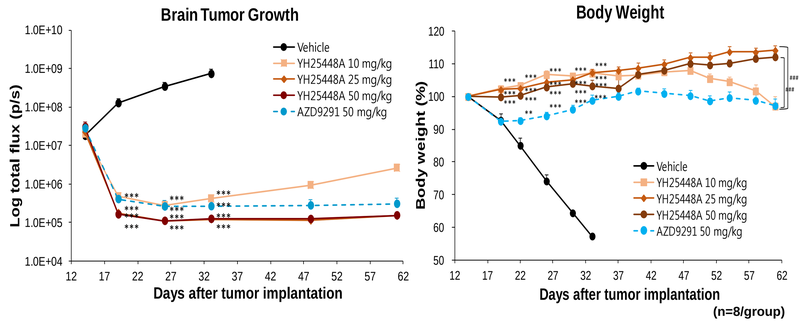
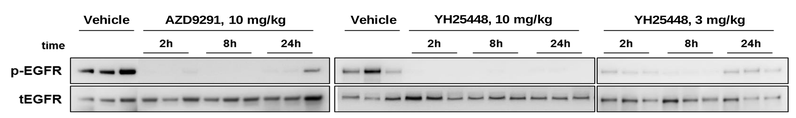


Fig. 6. Dose response efficacy of YH25448A (a salt form) in H1975 intracranial tumor model in BALB/c nude mice



High BBB penetration profile and clear PK/PD correlation was well translated into excellent efficacy in brain metastases model

Fig. 7. Pharmacodynamic activity of YH25448 in subcutaneous tumor tissue



* Tumor tissues were collected after 13 days once daily repeated oral administration of test drugs.

Fig. 8. PK/PD correlation and tumor/brain tissue distribution properties of YH25448

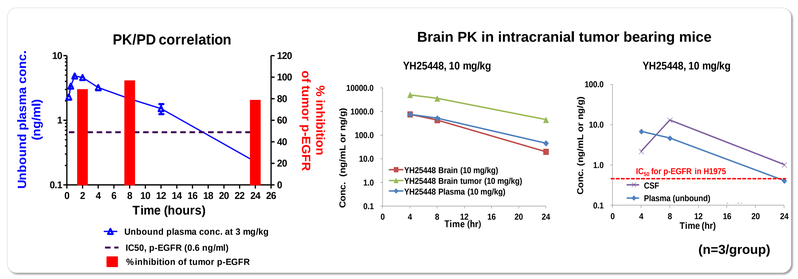
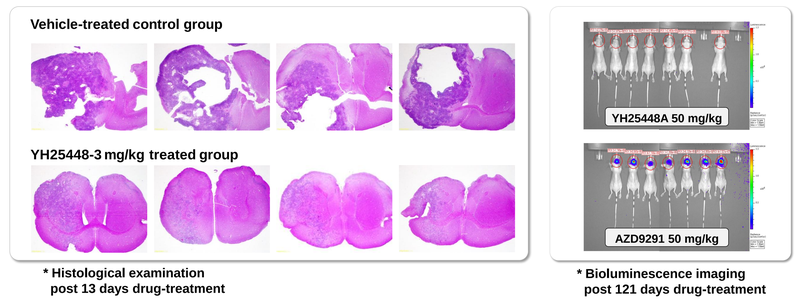


Fig. 9. Histological improvement of brain metastases by YH25448 and live imaging



* Histological examination post 13 days drug-treatment

* Bioluminescence imaging post 121 days drug-treatment

CONCLUSION

- ◆ **YH25448 shows dose dependent tumor growth inhibition and more potent and complete anti-cancer efficacy in both subcutaneous and intracranial tumor growth than AZD9291 with better tolerability at high dose level.**
- ◆ **High potency and high BBB penetration profile of YH25448 well translates into the greater survival benefit in brain metastases model of NSCLC.**
- ◆ **These findings provide rationale for the further development of YH25448 as a novel therapeutic for the treatment of EGFR mutant-positive patients. In particular, YH25448 will be able to address the urgent unmet needs for EGFR mutant-positive patients with brain metastases.**

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