YH25448, an irreversible 3rd-generation EGFR TKI, exhibits superior anticancer effects with potent brain BBB penetration in NSCLC

Jiyeon Yun[1], Min Hee Hong[2], Seok-Young Kim[1], Chae Won Park[1], So-Young Kim[1], Mi Ran Yun[1], Han Na Kang[1], Kyoung-Ho Pyo[1], Jong Sung Koh[4], Ho-Juhn Song[4], Young-Sung Lee[5], Se-Woong Oh[5], Soongyu Choi[5], and Byoung-Chul Cho*[1]

[1]Yonsei University College of Medicine, Cancer Research Inst., Seoul, Republic of Korea; [2]Yonsei University College of Medicine, Cancer Research Inst, Seoul, Republic of Korea; [3]Yonsei University College of Medicine. Cancer Research Inst., Seoul, Republic of Korea; [4]Genosco Inc., Cambridge, MA; [5]Yuhan R&D Institute, Yuhan Corporation, Seoul, Republic of Korea

ABSTRACT

Epidermal growth factor receptor (EGFR) mutations are present in approximately 10-15% of non-small cell lung cancers (NSCLC). Although therapeutic EGFR tyrosine kinase inhibitors (TKIs) targeting mutant EGFR, such as gefitinib and erlotinib, are used in the first line treatment of patients with advanced EGFR mutated NSCLC, acquired resistance to these drugs usually appears within 10-12 months of therapy, commonly due to the occurrence of a second EGFR mutation, T790M.

YH25448 is a highly mutant-selective and irreversible 3rd generation EGFR TKI that penetrates the blood-brain barrier (BBB), and targets the activating EGFR mutations Del19 and L858R, as well as the T790M mutation, while sparing wild type.

In NSCLC cell lines and primary cancer cells from patients harboring EGFR mutations, YH25448 more potently inhibits cancer cell growth and significantly increases tumor cell apoptosis compared to osimertinib.

In an *in vivo* mouse model implanted with H1975 cells, once-daily YH25448 treatment resulted in dramatic dose-dependent tumor regression in both subcutaneous and intracranial lesions with no abnormal signs such as skin keratosis. The plasma half life of YH25448 was 5.9-6.8 hr, while the tumor to plasma $AUC_{0\text{-last}}$ ratio was 3.0-5.1 in tumor-bearing mice. YH25448 also showed excellent penetration of the BBB, achieving CSF concentrations exceeding the IC_{50} value for pEGFR inhibition.

Taken together, these findings support the further development of YH25448 as a novel therapeutic for the treatment of EGFR mutant-positive NSCLC patients with brain metastases.

INTRODUCTION

First-line treatment with EGFR TKIs represents the standard of care for NSCLC patients with activating EGFR mutations. Real-world results have shown that median overall survival (OS) reaches about 31 months, while the 5-year OS rate is approximately 15% when metastatic EGFR-mutated NSCLC is treated with EGFR TKIs (1-2).

However, the majority of patients eventually relapse with disease progression within 1 to 2 years after receiving EGFR TKI therapy (3). For this reason, there have been many efforts to target the EGFR T790M mutation and third-generation EGFR TKIs including rociletinib, olmutinib, and osimertinib have been developed. Brain metastasis is present in one-quarter of patients at diagnosis, and about half of patients with EGFR-mutated lung cancer develop brain metastases within 3 years (4).

Despite the introduction of third-generation EGFR TKIs, significant unmet needs remain. Many of these agents elicit unintended side effects as they still target wild-type EGFR to some degree. Only osimertinib (AZD9291) has been approved for this indication by the U.S. FDA and the European Medicines Agency. However, even in a phase III trial of osimertinib, adverse events at all grades occurred in almost every patient (98%), and 23% of the patients experienced grade 3 or higher adverse events (5). The incidences of diarrhea and rash with osimertinib therapy at any grade were 41% and 34%, respectively. These adverse effects were considered to be caused by the low selectivity of osimertinib against WT EGFR. Another pitfall of the EGFR TKIs developed to date is their relative inability to penetrate the blood–brain barrier (BBB). The central nervous system (CNS) is one of the most common sites of progression during EGFR TKI treatment, and NSCLC patients with CNS metastasis have a very poor prognosis (6-8).

YH25448 is a novel, highly potent, and mutant-selective EGFR TKI with a wide therapeutic index and exceptional intracranial activity. To address the limitations of available third-generation EGFR TKIs, we report the kinome-wide screening data, cell line data, *in vivo* data obtained in mouse models with subcutaneous and brain implantation, and in a patient-derived model, as well as response data for the patients enrolled in an early clinical trial of YH25448. We have embarked on a phase I/II study (NCT03046992) of YH25448 in NSCLC patients with EGFR mutations who have failed to respond to prior EGFR TKI treatment.

RESULTS

1. YH25448 is a novel, irreversible inhibitor of EGFR kinase activity, selectively targeting mutant EGFRs.

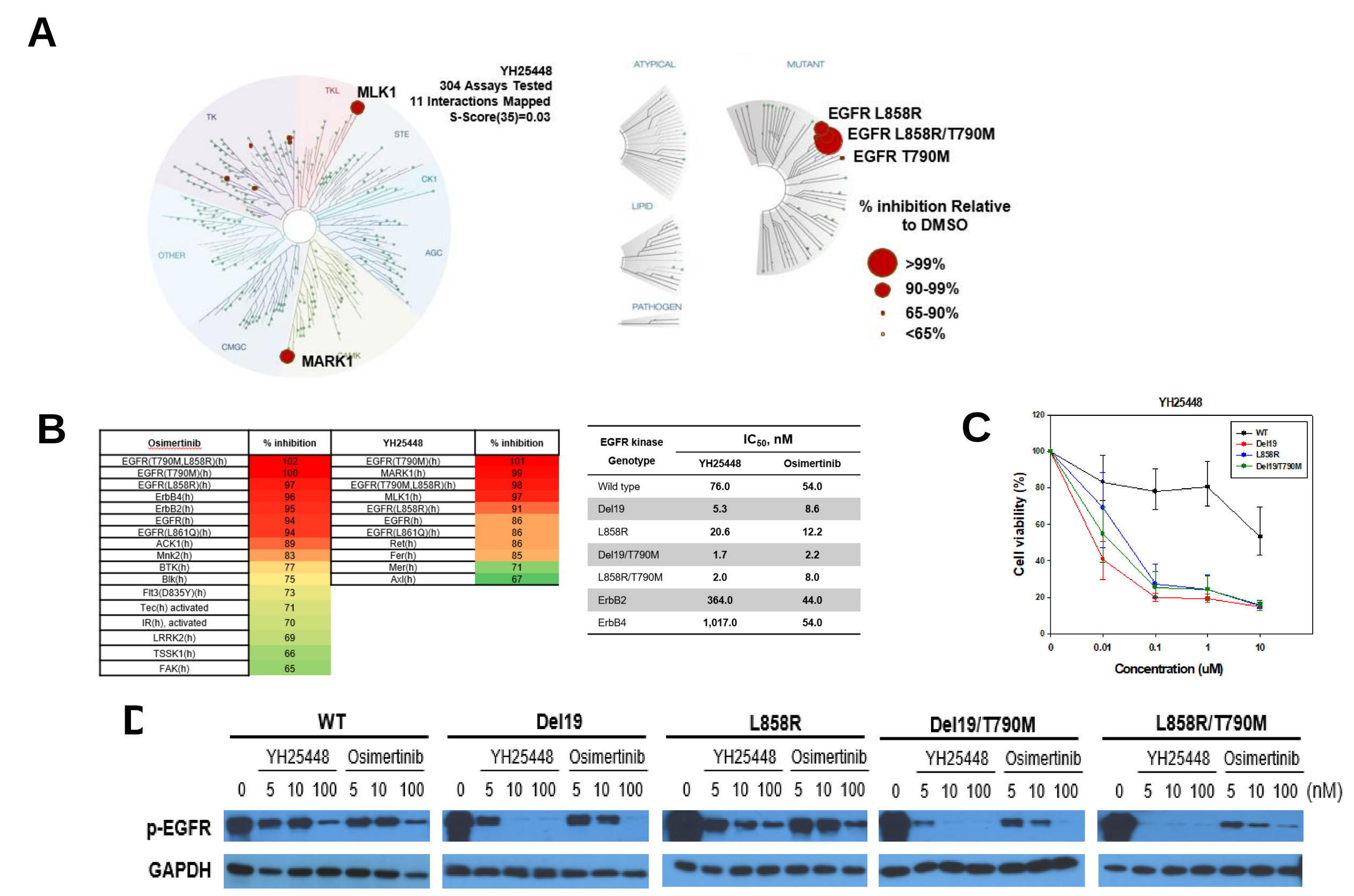

Figure 1. Characterization of the EGFR mutant-selective irreversible inhibitor YH25448. A, kinome tree for YH25448 generated using DiscoveRx TREEspot version 4. Sizes of the red circles are proportional to the percentage inhibition at the test concentration (1 μM): largest circle, 99% inhibition; medium circle, 90 to 99% inhibition; smallest circles, 65 to 90% inhibition. B, Comparison of YH25448 and osimertinib selectivity profiles against ~320 kinases. The kinases listed were subject to over 65% inhibition by each compound, compared to DMSO. C, viability of Ba/F3 cells was determined with an MTT assay. YH25448 was treated for 72 hrs. D, Ba/F3 cells overexpressing the indicated EGFR mutant were treated with YH25448 or osimertinib for 6 hours at the indicated concentrations. pEGFR levels were detected by Western blot analysis.

2. YH25448 significantly regresses lung cancer with EGFR mutations *in vitro* and *in vivo.*

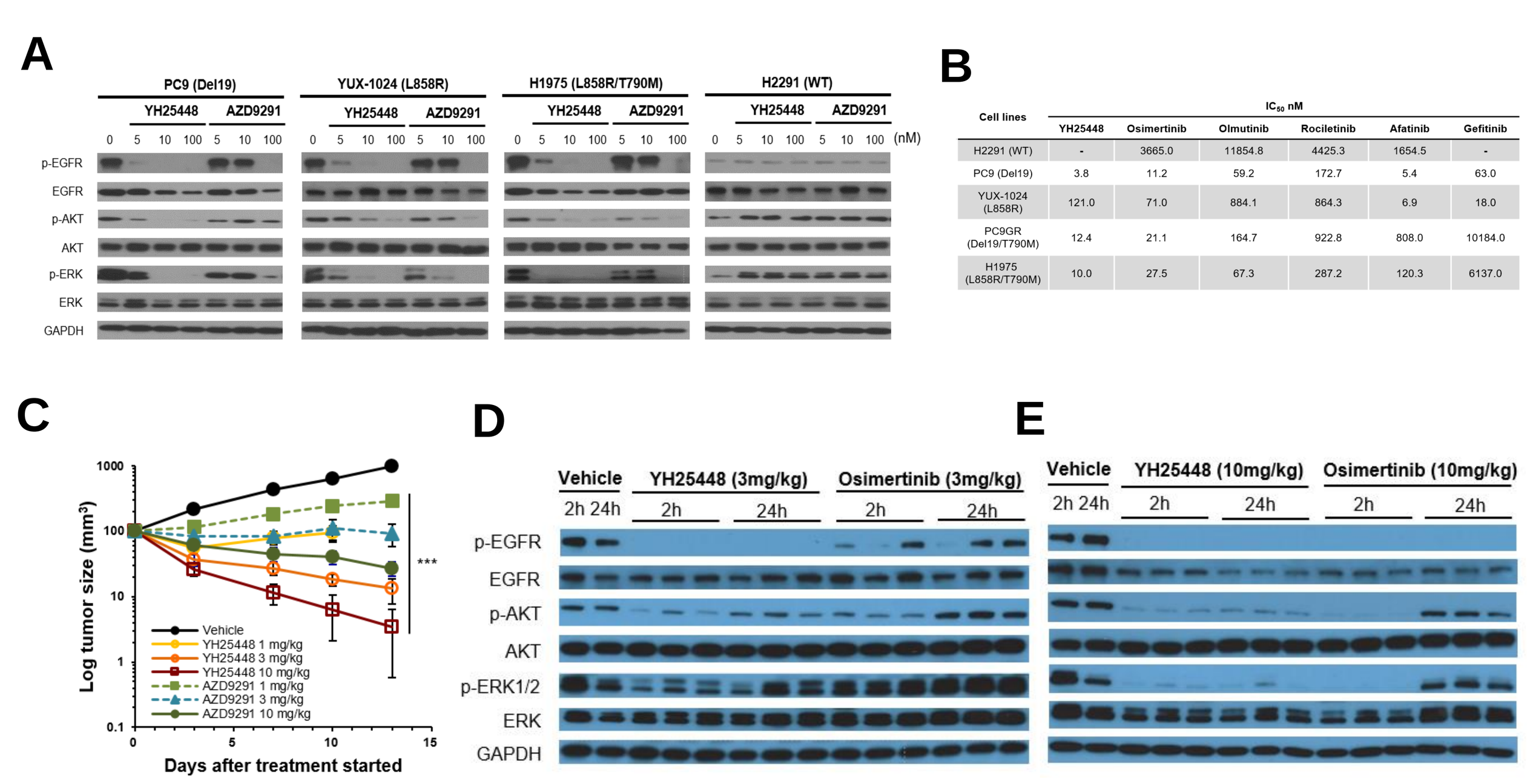

Figure 2. Potency and selectivity of YH25448. A, Immunoblot analysis was performed for EGFR, AKT, and ERK expression after YH25448 or osimertinib treatment for 6 hours at the indicated concentrations in lung cancer cell lines. B, IC_{50} values for the indicated EGFR TKIs in the cells (treatment time = 72 h). C, Antitumor effects of YH25448 in H1975 (L858R/T790M) tumor-bearing mice (n = 7/group). Mice were treated with YH25448, osimertinib, or vehicle once daily for 2 weeks after the tumor volume reached 100 mm³. Data represent the mean ± SEM (n = 7/group). ***P < 0.001 *vs.* vehicle control. D-E, Tumor lysates of vehicle-, YH25448-, or osimertinib-treated H1975 xenograft mice for 3 days were harvested at the indicated time point after the last treatment. Lysates were subjected to immunoblotting for pEGFR (Y1068), p-AKT, and p-ERK1/2.

3. YH25448 shows superior efficacy for tumor regression in an EGFR mutant brain metastasis model, with a high BBB penetration profile.

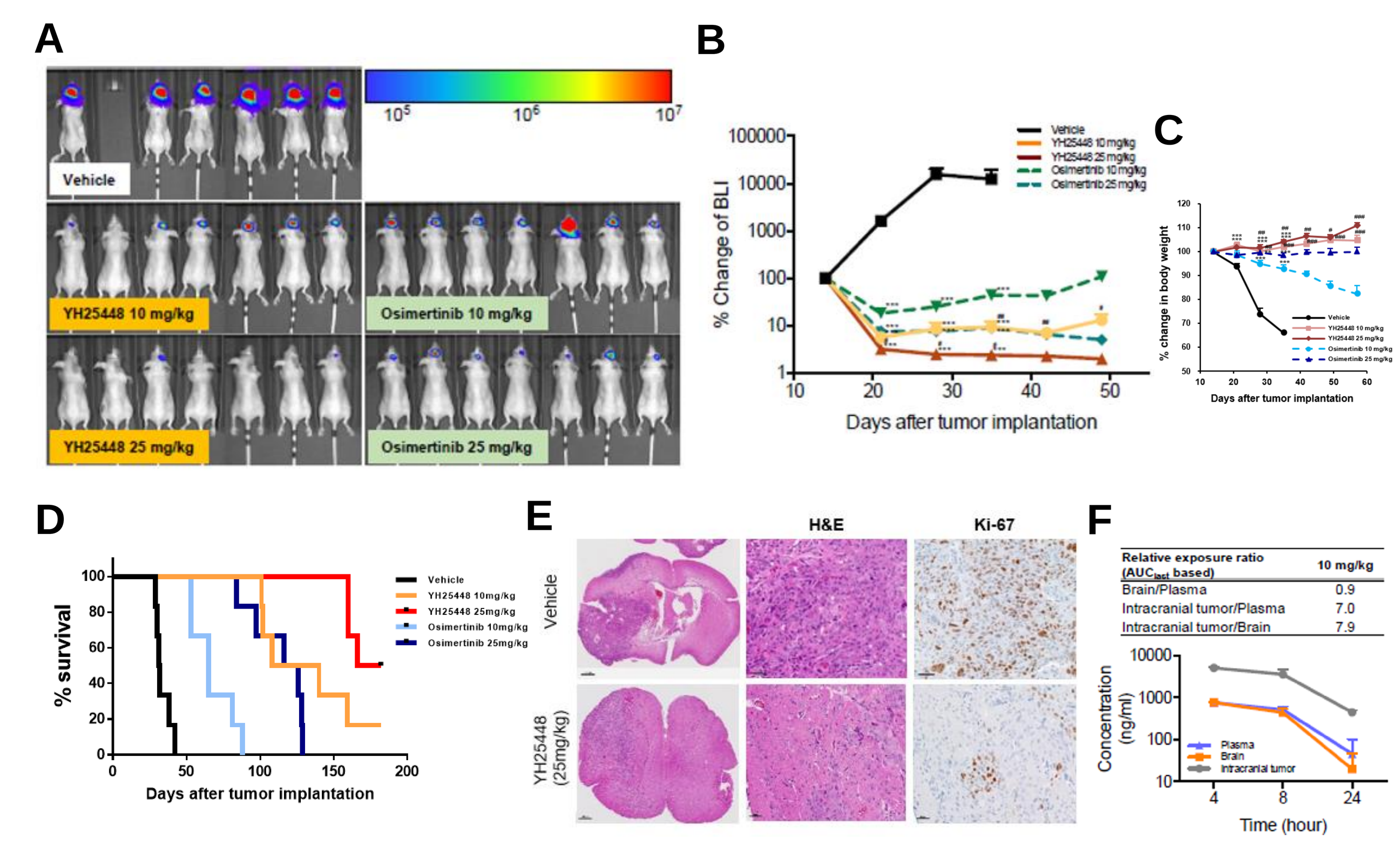

Figure 3. Intracranial anti-tumor effects of YH25448 in the H1975-luc brain metastasis model. A-B, An intracranial tumor growth model was established with BALB/c nude mice using H1975-luc cells. Two weeks after H1975-luc injection, animals were treated with YH25448 or osimertinib once daily. Bioluminescence intensity (BLI) was used to detect intracranial tumor growth in vivo. Data represent the mean ± SEM (n = 7/group). ***P < 0.001 *vs.* vehicle control; #P < 0.01, ##P < 0.05 *vs.* osimertinib. C, Body weight. Bars, Data are presented as mean±S.E.M. (n=7/group) **, p<0.01; ***, p<0.001 vs. vehicle control, #, p<0.05; ##, p<0.01; ###, p<0.001 vs AZD9291 at the same dose. D, Kaplan–Meier survival curves of the animals. E, Histopathological examination of brain sections obtained following H1975-luc intracranial implantation. Scale bars, 25 μm. F, Plasma, intracranial tumor, and brain tissue samples obtained at 4, 8, and 24 hours post dosing of YH25448 (10 mg/kg) on day 21 post-dose were analyzed with a validated LC/MS/MS method.

4. YH25448 elicits potent antitumor activity in an EGFR mutant patient-derived tumor xenograft model and patients with the T790M mutation.

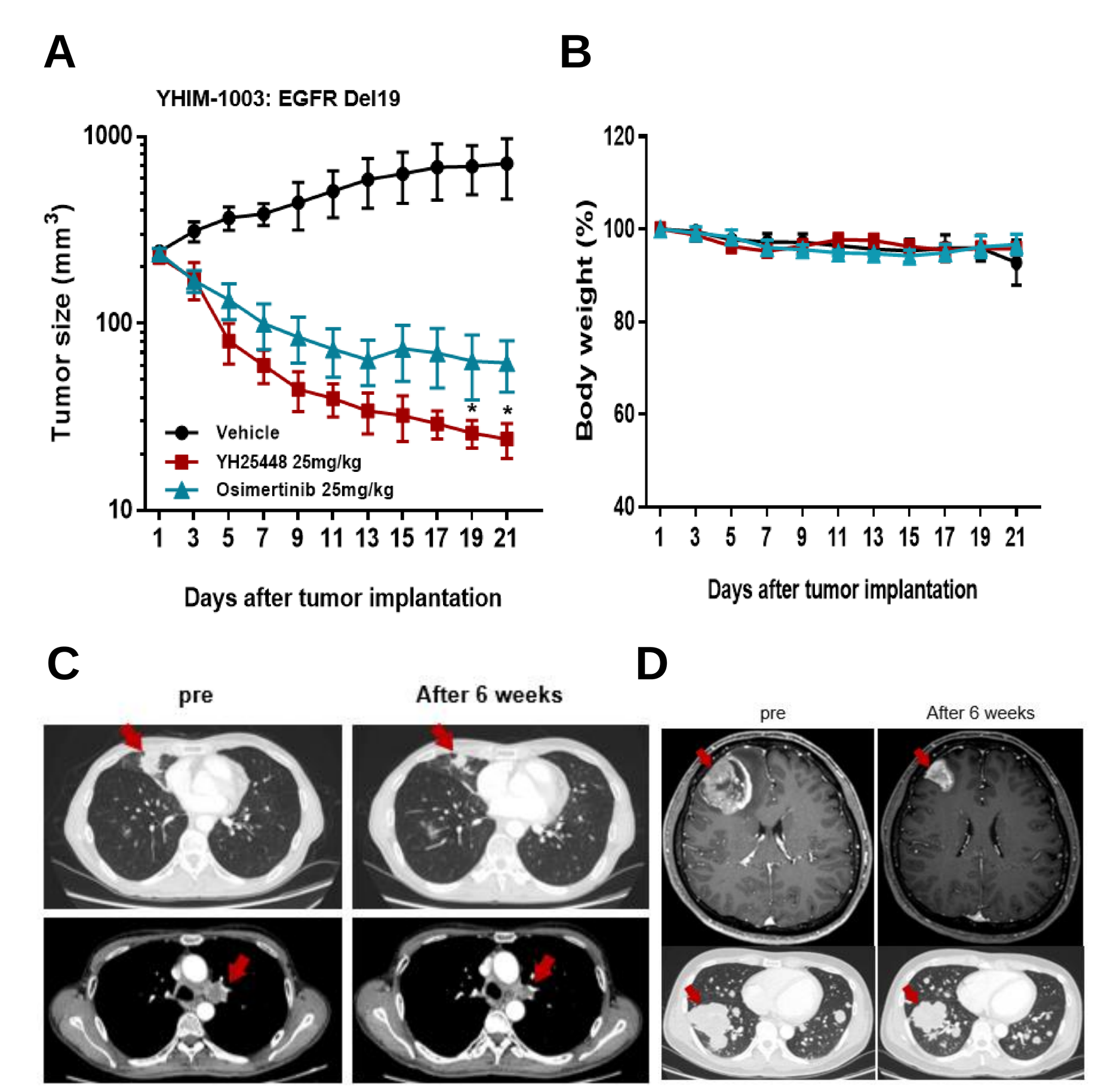

Figure 4. Efficacy of YH25448 in a patient-derived xenograft mouse model and representative response in the patients. A, Patient-derived tumors implanted in mice (YHIM-1003; EGFR ex19Del; passage 3) were treated with YH25448 or osimertinib at 25 mg/kg, q.d., when tumors reached 200 mm³ in volume. Data represent the mean ± SEM (n = 7/group). *P < 0.01 vs. osimertinib. B, Body weight. C, Radiologic response following YH25448 20 mg treatment in a 62-year old male patient with the EGFR T790M mutation. D, Intracranial and extracranial response after YH25448 40 mg treatment in a 37-year old male patient with the EGFR T790M mutation. Red arrow indicates the tumor lesion.

5. YH25448 shows greater selectivity against wild-type EGFR results in lower suppression of wild-type pEGFR in skin and lower skin toxicity than osimertinib in animals.

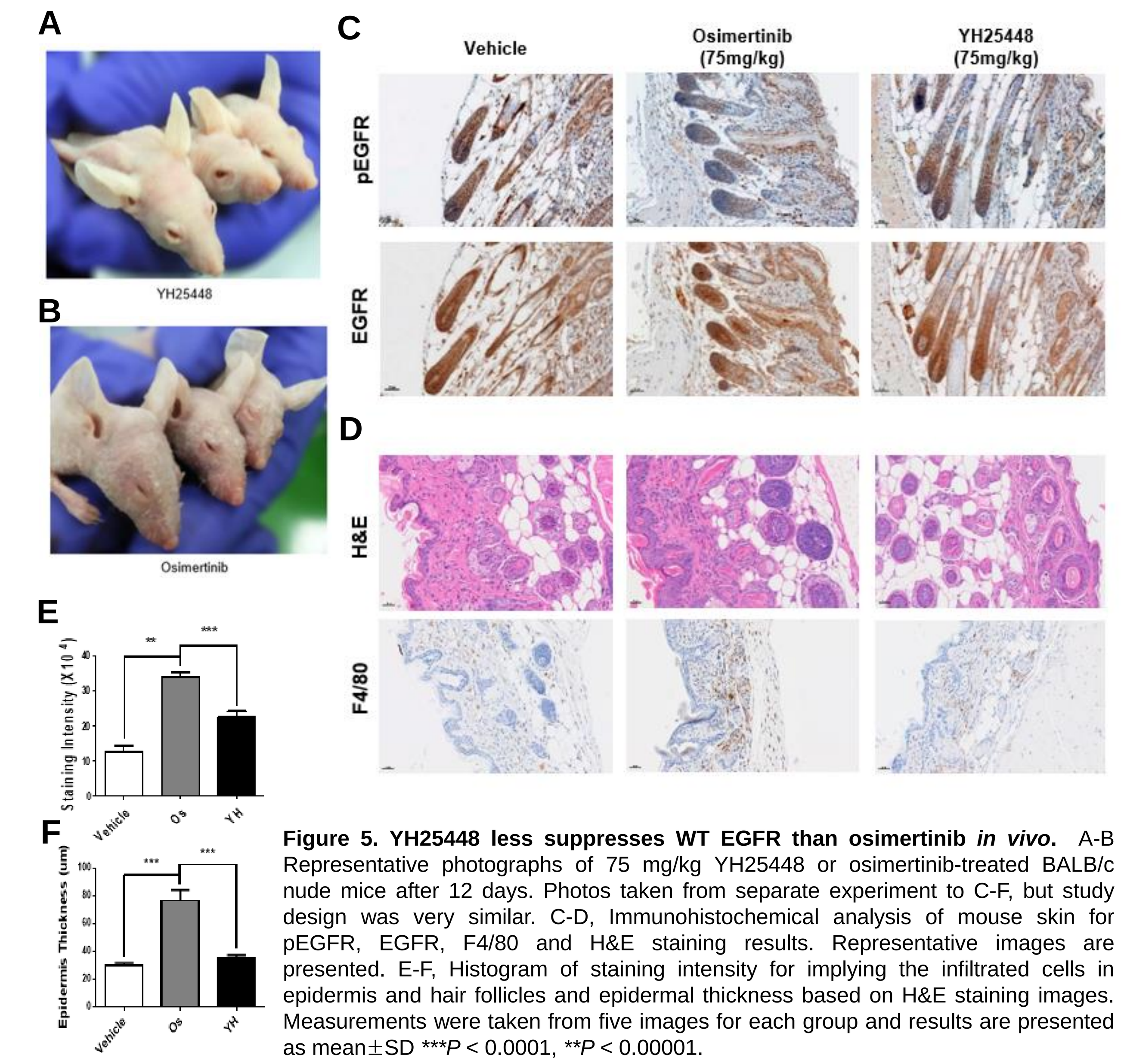

Figure 5. YH25448 less suppresses WT EGFR than osimertinib *in vivo*. A-B Representative photographs of 75 mg/kg YH25448 or osimertinib-treated BALB/c nude mice after 12 days. Photos taken from separate experiment to C-F, but study design was very similar. C-D, Immunohistochemical analysis of mouse skin for pEGFR, EGFR, F4/80 and H&E staining results. Representative images are presented. E-F, Histogram of staining intensity for implying the infiltrated cells in epidermis and hair follicles and epidermal thickness based on H&E staining images. Measurements were taken from five images for each group and results are presented as mean±SD ****P* < 0.0001, ***P* < 0.00001.

CONCLUSIONS

YH25448 has demonstrated important competitive advantages in nonclinical studies

- Superior *in vitro* potency and selectivity over osimertinib.
- Superior *in vivo* efficacy over osimertinib in both single (del19, L858R) and double (L858R/T790M) mutant xenograft models.
- Excellent BBB penetration and superior *in vivo* efficacy over osimertinib in a brain metastasis model.
- Lower skin toxicity over osimertinib.

Phase I/II clinical study in EGFR mutant-positive NSCLC patients are underway (NCT03046992)

- Representative cases presented here demonstrate promising anti-tumor efficacy for both intracranial and extracranial lesions

YH25448 is a promising novel 3rd generation EGFR TKI for patients with EGFR sensitizing or T790M resistant mutations

REFERENCES

(1) Novello S, Barlesi F, *et al.* Metastatic non-small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol **2016**;27:v1-v27
(2) Janne PA, *et al.* AZD9291 in EGFR inhibitor-resistant non-small-cell lung cancer. The New England journal of medicine **2015**;372:1689-99
(3) Camidge DR, *et al.* Acquired resistance to TKIs in solid tumours: learning from lung cancer. Nature reviews Clinical oncology **2014**;11:473-81
(4) Rangachari D, *et al.* Brain metastases in patients with EGFR-mutated or ALK-rearranged non-small-cell lung cancers. Lung cancer (Amsterdam, Netherlands) **2015**;88:108-11
(5) Passaro A, *et al.* Targeting EGFR T790M mutation in NSCLC: From biology to evaluation and treatment. Pharmacological research **2017**;117:406-15
(6) Togashi Y, *et al.* Cerebrospinal fluid concentration of gefitinib and erlotinib in patients with non-small cell lung cancer. Cancer chemotherapy and pharmacology **2012**;70:399-405
(7) Heon S, *et al.* Development of central nervous system metastases in patients with advanced non-small cell lung cancer and somatic EGFR mutations treated with gefitinib or erlotinib. Clin Cancer Res **2010**;16:5873-82
(8) Ali A, *et al* . Survival of patients with non-small-cell lung cancer after a diagnosis of brain metastases. Current oncology (Toronto, Ont) **2013**;20:e300-6

Abstract No. 4790 | AACR Annual meeting 2018 | April 14-18, Chicago

For more information, please contact us at:
hanjookim@yuhan.co.kr , +82-31-899-4203, or visit www.yuhan.co.kr