약학석사학위논문

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Qiu Yu 의 석사학위논문을 인준함

2018 년 8 월

위 원 장 _____ (인)

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위 원 _____ (인)

Abstract

Simultaneous inhibition of c-MET and EGFR for overcoming resistance to EGFR-therapy in NSCLC

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Mesenchymal-epithelial transition factor (MET)，the only high affinity receptor for hepatocyte growth factor (HGF), plays an important role in human carcinoma and is recognized as a therapeutic target. In addition, c-Met and EGFR are receptor tyrosine kinases that may cross-talk in driving the development and progression of non–small cell lung cancer (NSCLC). In this study, it described the small molecular compound ABN401, a novel inhibitor of MET kinase, and the combination therapy with EGFR tyrosine kinase inhibitors, including Erlotinib, Afatinib and Tagrisso. The results from this study show that this inhibitor potently blocks MET phosphorylation and activation of its key downstream

effectors especially in MET amplified lung cancer cell lines. MET amplified mouse tumor models also show dose-dependent inhibition of tumor growth. In a further exploration of potential treatment for MET activation and signaling through intracellular signaling cascade enhanced by EGFR, the results showed that combination treatment with EGFR inhibitors positively repress tumor growth compared to single treatment both *in vitro* and *in vivo*. This study suggests that ABN401 is a potent and selective MET inhibitor that may have therapeutic potential in lung cancer treatment. Moreover, development of combination therapies by utilizing MET and EGFR inhibitors may greatly improve treatment effect in lung cancer patients.

Keywords: MET amplification, EGFR-therapy, combination therapy, NSCLC

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LIST OF ABBREVIATIONS

RTK	Receptor tyrosine kinase
NSCLC	Non-small cell lung cancer
MET	Mesenchymal epithelial transition factor
HGF	Hepatocyte growth factor
EGFR	Epidermal growth factor receptor
IC50	50% inhibitory concentrations
CI	Combination index
PDX	Patient-derived xenograft
TGI	Tumor growth inhibition

INTRODUCTION

MET receptor tyrosine kinase (RTK) signaling, driven by its unique biological ligand hepatocyte growth factor (HGF), is a key signaling pathway which is not only important for many normal development and homeostatic processes but is also responsible for the pathologic development and progression of many human cancers [1]. The regulation of proliferation, apoptosis and migration mediated by MET signaling pathway through overexpression, amplification or mutation of the MET gene has been widely confirmed in oncogenic processes in various tumor types and has been reviewed before [2-5]. In addition, it is noteworthy that all of these mechanisms of dysregulation have been found in non–small cell lung cancer (NSCLC) [2,6-8], which accounts for more than 80% of the cases of lung cancer. Therefore, the MET pathway has become an attractive target for the treatment of non-small cell lung cancer.

Like MET, overexpression and mutation in the epidermal growth factor receptor (EGFR) are also considered as promising biomarker for the treatment of NSCLC by tyrosine kinase inhibitor (TKIs) [9]. Although the clinically approved TKIs such as Gefitinib and Erlotinib can produce impressive responses in cancer patients' treatment, rapidly resistance remains a significant limitation to the long-term efficacy of

such treatments [10-12]. There are two main reasons. First, the mutation frequency of *EGFR* gene is very high, and most patients develop drug resistance like T790M mutation after treatment with EGFR TKIs. To overcome the drug resistance mechanism, the second (Afatinib) and third (Tagrisso) generation TKIs have been developed and applied in clinical therapy. Another reason is availability of other pathways, such as MET, which can bypass EGFR signals to maintain survival and growth of tumor cells. Based on the co-expression of MET and EGFR, which plays as an essential resistance mechanism for EGFR-targeted therapy, it is essential to establish mechanisms of resistance and to apply that knowledge to the development of strategies to combat resistance [13]. One such strategy is that targeting MET and EGFR at the same time might enhance the anti-tumor effect and provide better treatment options for NSCLC patients.

Here, it described a newly developed and highly selective c-Met inhibitor, ABN401, that exhibited strong potency against MET kinase, repressed MET phosphorylation and the downstream signal transduction in various MET–aberrant lung cancer cell lines especially in EBC-1, which harbors high MET amplification. In addition to the listed, ABN401 manifested dramatic antitumor activity in MET high amplification-driven xenograft models at tolerable doses.

To further validate the synergistic efficacy of MET and EGFR inhibition simultaneously against NSCLC with multiple aberrant expressions, I investigated the effects of ABN401 and EGFR-TKIs on

in vitro cell proliferation, signal transduction and on *in vivo* tumor growth of NSCLC. The results suggested that combined targeting MET and EGFR pathways can provide dramatic synergistic inhibitory effects, pointing to a potential therapeutic strategy to reduce the possibility of relapse in NSCLC patients with EGFR-therapy resistance and emphasizing the close relationship between MET and EGFR signals in NSCLC.

Materials and Methods

Chemicals

ABN401 was obtained from Abion (Korea). Erlotinib (S7786), Afatinib (S1011), Tagrisso (S7297) were purchased from Selleck Chemicals (USA). All compounds were reconstituted in 100% DMSO to a 10 mM concentration and stored at −80°C.

Cell lines and cell culture

Cell line EBC-1 was purchased from the Japanese Collection of Research Bioresources Cell Bank (JCRB Cell Bank, Japan). Cell lines H820 and H1993 were purchased from the American Type Culture Collection (ATCC, USA), and cell lines H596 and H1373 were obtained from the Korean Cell Line Bank (KCLB, Korea). EBC-1 was cultured in EMEM (Hyclone, USA) supplemented with 10% FBS (Hyclone, USA), 1% penicillin/streptomycin solution (Hyclone, USA). H820 was cultured in RPMI (Hyclone, USA) supplemented with 5% FBS (Hyclone, USA), 1% penicillin/streptomycin solution (Hyclone, USA). Other cell lines were cultured in RPMI (Hyclone, USA) supplemented with 10% FBS (Hyclone, USA), 1% penicillin/streptomycin solution (Hyclone, USA). Cells were passaged once every 2~3 days at a confluence of 70~80%. All of the cells were grown at 37℃ in a humidified incubator under 5% CO_2.

Cell viability assay

All cells were seeded in 96-well plates, 5000–8000/well, in growth media overnight and then exposed to increasing concentrations (range, 1–1000 nM) of ANB401 for 72 hours at 37℃. After adding WST solution (DoGen, Korea) and incubating for 2-3 hours, the number of viable cells was determined at 450 nm using a multi-reader (Tecan, Switzerland) and expressed as a percentage of viable cells, considering the untreated control cells as 100%. At least three independent experiments were performed in triplicates and data were pooled.

Chou and Talalay analysis

H1373 was treated with ABN401 and Erlotinib, and H820 was treated with ABN401 and Afatinib or Tagrisso in dose dependent manner and synergistic inhibition was measured by using WST assay at a 1:1 ratio of each drug. To determine the pharmacological interaction of combination treatment, the Chou and Talalay analysis was used [14]. This method assesses synergism and antagonism by quantifying the divergence of the combination effect from the expected additive effect of the two therapeutic agents. A combination index (CI) was estimated from the dose-effect data. A CI of >1, =1 and of <1 indicates antagonism, additive effects and synergism respectively.

Western blot analysis

Briefly, all cells were treated with increasing concentrations (range, 1–10000 nM) of ANB401. H1373 was treated with ABN401 (3 μM) and Erlotinib (4 μM) alone and in combination of both. H820 was treated with ABN401 (2 μM), Afatinib (50 nM) and Tagrisso (500 nM) respectively and combination of ABN401 (2 μM) and Afatinib (50 nM) or ABN401 (2 μM) and Tagrisso (500 nM). After 72 hours incubation, cells were harvested with lysis buffer containing RIPA (Biosesang, Korea), protease inhibitor (Roche, USA), and phosphatase inhibitor (Roche, USA) and lysed using 1 ml syringes. Protein concentration was measured with BCA assay kit (Thermoisher, USA). The protein solution was boiled with 5X SDS sample loading buffer for 7 minutes to denature the proteins. The proteins were separated according to molecular weight on 8% SDS-polyacrylamide gel and transferred onto polyvinyl difluoride (PVDF) membrane, which was blocked with 5% BSA in 1X Tris—buffered saline with 0.1% Tween-20. The membrane was incubated with primary antibody MET antibody (Cell signaling, USA, #8198), p-Met antibody (Tyr1234/1235) (Cell signaling, USA, #3077), p-Met (Tyr1349) antibody (Cell signaling, USA, #3133), EGFR antibody (Cell signaling, USA, #2232), p-EGFR antibody (Tyr1068) (Cell signaling, USA, #2220), Akt antibody (Cell signaling, USA, #4691), p-Akt (s473) antibody (Cell signaling, USA, #4060), p44/42 MAPK (Erk1/2) antibody (Cell signaling, USA, #4695), p-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) antibody (Cell signaling,

USA, #4370), β-actin antibody (Santa Cruz Bioechnology, sc-47778) in 1X TBS-T with 1% BSA, followed by incubation with a secondary antibody (anti-rabbit and anti-mouse horseradish peroxidase-conjugated antibody, Thermoisher, USA). At each step, the membrane was rinsed with 1X TBS-T three times for a total 30 minutes. After the last rinse, the membrane was incubated with ECL (Amersham, USA) and images were captured with ImageQuant LAS 4000 (GE Healthcare, Chalfont St. Giles, UK).

Tumor xenograft models

The *in vivo* anti-tumor efficacy of ABN401 was tested in EBC-1 and H1373 xenograft models. All animal experiments were performed according to the Seoul National University Institutional Animal Care and Use Committee (SNU-150720-3). Tumor cells (EBC-1 & H1373) were harvested and resuspended in serum-free MEM or RPMI. Six-week-old female athymic nude mice (Koatech, Pyeongtaek, Korea) were injected subcutaneously at the right flank with viable cells at 2×10^6 cells/mouse. Following cell injection, when average tumor volume reached to approximately 200 mm^3 (EBC-1) and 400 mm^3 (H1373), the tumor size was measured twice weekly with digital calipers and tumor volumes were calculated as the formula:

$$V = [\text{long diameter} \times \text{short diameter}^2] \times 0.5.$$

***In vivo* drug efficacy evluation on xenograft tumor**

To test the efficacy of ABN401 on EBC-1 cell-derived xenograft tumor growth, 20 tumor-bearing mice for each mouse strain were randomized into four groups (5 mice/group) for the following treatments: group 1, vehicle; group 2, ABN401 0.04 mg/mouse (2 mg/kg); group 3, ABN401 0.2 mg/mouse (10 mg/kg); and group 4, ABN401 0.6 mg/mouse (30 mg/kg). The ABN401 was dissolved in 20% polyethylenglycol (PEG) 400 (Sigma, USA) in 0.1 M acetate buffer pH4. Treatments were started when the average tumor size reached 200mm^3, and drugs were administered for 5 days continuously and 2 days off through oral gavage for 3 weeks. The treatment was stopped until the tumor size reached to 2000 mm^3. The tumor sizes were monitored and measured twice weekly.

In the case of combined efficacy of ABN401 and Erlotinib on H1373 cell-derived xenograft tumor growth, 20 tumor-bearing mice for each mouse strain were randomized into four groups (5 mice/group) for the following treatments: group 1, vehicle; group 2, ABN401 0.6 mg/mouse (30 mg/kg); group 3, Erlotinib 0.6 mg/mouse (30 mg/kg); and group 4, ABN401 0.6 mg/mouse (30 mg/kg) plus Erlotinib 0.6 mg/mouse (30 mg/kg) combination. Both ABN401 and Erlotinib were dissolved in 20% polyethylenglycol (PEG) 400 (Sigma, USA) in 0.1 M acetate buffer pH4. Treatments were started when the average tumor size reached 400mm^3, and drugs were administered for 5 days continuously and 2 days off through oral gavage for 3 weeks. The treatment was stopped until the tumor size reached to 2000 mm^3. The tumor sizes were monitored and measured twice weekly.

PDX model establishment

In vivo efficacy tests were performed in female BALB/c-nude mice bearing human primary lung tumor tissue to treat different concentration of ABN401 or in combination with Erlotinib on PDX NSCLC model. Tumors from stock mice bearing human primary lung tumors were harvested, dissected into fragments and inoculated into the 10-week-old female BALB/c nude mice. Each mouse was inoculated subcutaneously at the right flank with tumor fragment with 2–4 mm sizes for tumor development. When average tumor size reached approximately 150 mm^3, mice were randomly allocated into 5 groups, with 8 mice per group, and treated with vehicle, ABN401 10 mg/kg, ABN401 30 mg/kg, Erlotinib 30 mg/kg, ABN401 30 mg/kg plus Erlotinib 30 mg/kg, respectively. Both ABN401 and Erlotinib were dissolved in 20% polyethylenglycol (PEG) 400 (Sigma, USA) in 0.1 M acetate buffer pH4. Drugs were administered for 5 days continuously and 2 days off through oral gavage for 3 weeks. The tumor size was measured twice weekly with digital calipers and tumor volumes were calculated as the formula:

$$V = [\text{long diameter} \times \text{short diameter2}] \times 0.5.$$

Statistical analysis

Student t-test was used to analyze the statistical significance of the data. All statistical analyses in this study were performed using GraphPad

Prism (GraphPad Software, USA) and p-value less than 0.05 was considered statistically significant.

RESULTS

The efficacy of ABN401 in various MET aberrant expression types

First, the effect of ABN401 on the proliferation *in vitro* of lung cancer cells harboring different aberrant *MET* expressions were examined. EBC-1 with high *MET* amplification was most sensitive to ABN401, with IC50 values of ≤10 nM (Table 1). In contrast, ABN401 showed less anti-prolierative effect on cell lines not only harboring *MET* aberrant expression but also affected by other oncogene drivers such as EGFR (Table 1, Figure 1). In order to confirm the effect of ABN401 on MET signal patterns, these cells were treated with increasing concentration of ABN401. MET and its downstream signaling was examined by western blot (Figure 2). The results showed that ABN401 has a dose-dependent inhibitory effect on MET phosphorylation, with a obvious supression at 1 μM in all tested cells. However, inhibition of the phosphorylation of Akt and Erk, which are the key downstream molecules of MET and play crucial roles in MET function [15,16], only markedly showed in EBC-1with *MET* high amplification (Figure 2E). To assess the *in vivo* activities of ABN401, EBC-1 cell–derived mouse tumor model was established. Mice were orally administered with increasing doses of ABN401, and it showed strong inhibition tumor growth in a dose-dependent manner (Figure 3). In conclusion, these

data indicated that ABN401 is an effective and highly selective MET kinase inhibitor for MET amplified NSCLC treatment.

ABN401 plus Erlotinib strengthens inhibition of H1373-dervived tumor growth

Next, I questioned the synergistic efficacy of MET and EGFR inhibition simultaneously on those cells with multiple aberrant expressions. First, I chosed cell line H1373 which shows strong co-expression of MET and EGFR and treated it with ABN401 and Elrotinib alone and a comnination with both. Acorroding to the cell viability, cells did not respond well to ABN401 or Erlotinib alone but showed notable sensitivity to the combination treatmentall. Also , all of the CI values were less than one, which indicated synergistic efficacy of combination treatment (Table 2, Figure 4). In the Western blot analysis, although the inhibition of the phosphorylation of EGFR by Erlotinib (4 μM) did not seem to synergize with ABN401, the suppression of phosphorylation of MET and downstream Akt and Erk was clearly enhanced by the combination of two drugs (Figure 5). To evaluate the antitumor efficacy of the combination of ABN401 and Elrotinib, H1373 tumor xenograft model was established. Although single treatment led to a slight decrease in tumor growth, combined treatment resulted in significant growth inhibition (Figure 6).

ABN401 treatment enhances sensitivity to EGFR-TKIs in H820 cells

To further explore the potential utility of combining MET and EGFR TKIs in the treatment of NSCLC with EGFR-therapy resistence, I focused on experiments of cell line H820, which naturally harbors EGFR exon19 deletion and T790M mutation as well as MET amplification [17]. Afatinib and Tagrisso were chose whose targets are EGFR exon19 deletion and T790M mutation respectively. As the results showed, combined targeting of MET and EGFR may provide better anti-proliferative activity against NSCLC, and the CI values also suggested synergism (Table 4,5 Figure 7). The western blot results showed that inhibition of either EGFR or MET kinase activity results in only partial inhibition of prosurvival signaling pathways. However, combined ABN401 and EGFR-TKIs may overcome EGFR-therapy resistance via the inhibition of the phosphorylation of MET, Akt, and Erk, as well as moderate EGFR phosphorylation, which was maintained in the presence of EGFR inhibitor alone (Figure 8).

***In vivo* antitumor effcacy of ABN401 with or without EGFR-TKIs in PDX tumor models**

To test the potential benefit of ABN01 treatment *in vivo*, human NSCLC PDX tumor model with EGFR mutation and *MET* amplification were established and applied. ABN401 as a single agent treatment at 10 or 30 mg/kg, 5D+/2D-, demonstrated dose-related anti-

tumor activity, with tumor growth inhibition (TGI) values of 68.6% and 84.9% on day 21, respectively; the difference in anti-tumor activity was both statistically significant when compared with vehicle control ($P<0.001$ and $P<0.001$). Furtermore, the combination of ABN401 (30 mg/kg, 5D+/2D-) with Erlotinib (30 mg/kg, 5D+/2D-) showed major anti-tumor activity, with TGI value of 90.1% on day 21, which had significant difference compared with vehicle control ($P<0.001$) (Figure 9).

Table 1. Mutation status and the IC50 of ABN401 in NSCLC cell lines

Cell lines	ABN401 IC50 (nM)	MET status	EGFR status	Other status	Reference
H1373	5075	Constitutive activation	Co-express with MET	Her2/3 overexpression KRAS mutation G12C	[18,19]
H820	2132	Amplification	Exon 19 deletion & T790M		[20]
H596	897	Exon 14 deletion	Overexpression	PI3K mutation E545K	[21]
H1993	43	Amplification	Co-expression	Positive c-Myc ErbB3 expression	[18]
EBC-1	2	Amplification	Co-expression		[18]

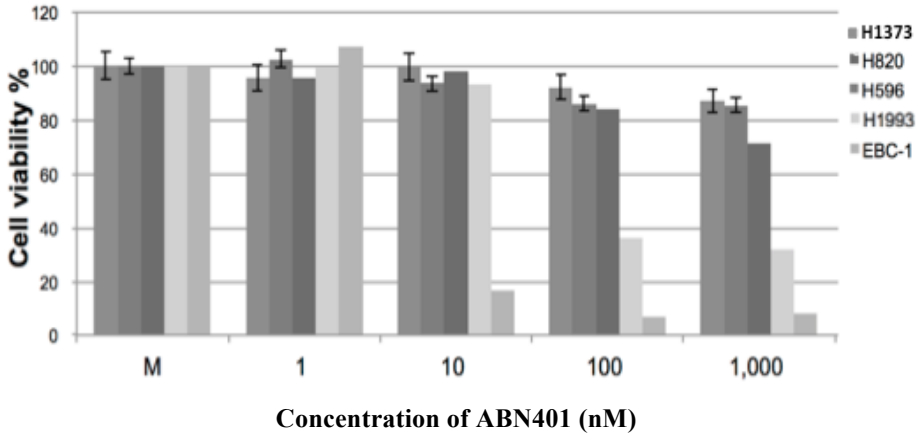
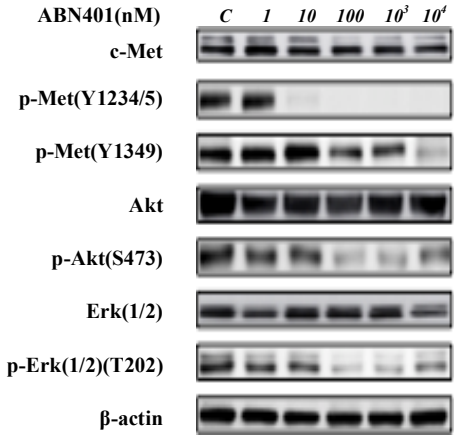


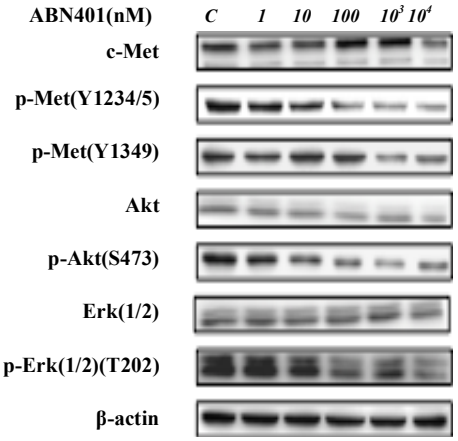
Figure 1. Sensitivities to ANB401 in MET aberrant NSCLC cell lines

Cell lines (H1373, H820, H596, H1993, EBC-1) were seeded in 96 well plaets with an increasing concentration of ABN401. The cells were incubated for 72 hours after teatment, and cell viability was assessed by WST assay.

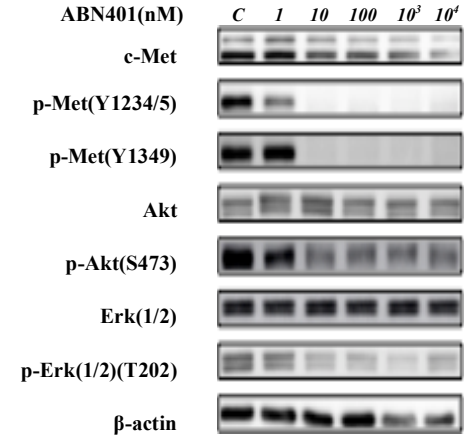
H1373



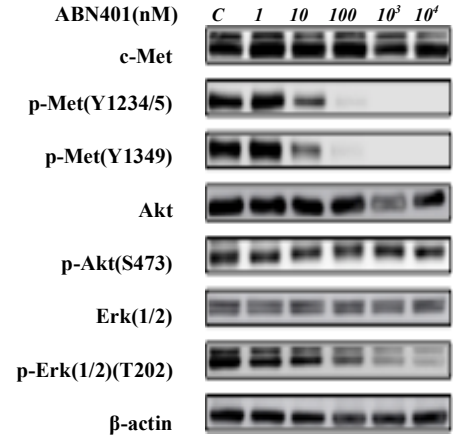
H820



H596



H1993



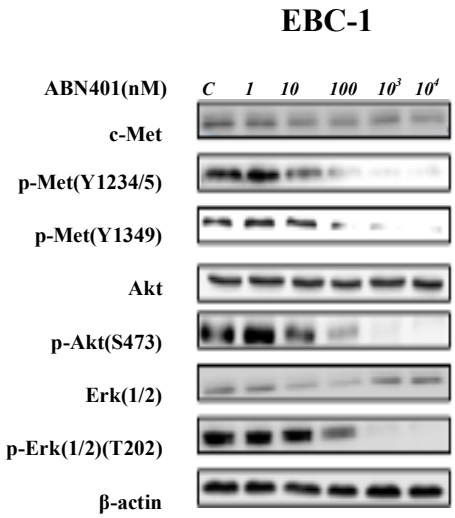


Figure 2. Effect of ABN401 on MET signal patterns in NSCLC cell lines

To verify the MET signal patterns, western blot was performed. Cell lines (H1373, H820, H596, H1993, EBC-1) were incubated with an increasing concentration of ABN401(1-10000 nM) for 72 hours, after which cell lysates were prepared and subjected to western blot analysis with antibodies to phosphorylation or total form of MET, Akt, or Erk and β-actin (loading control).

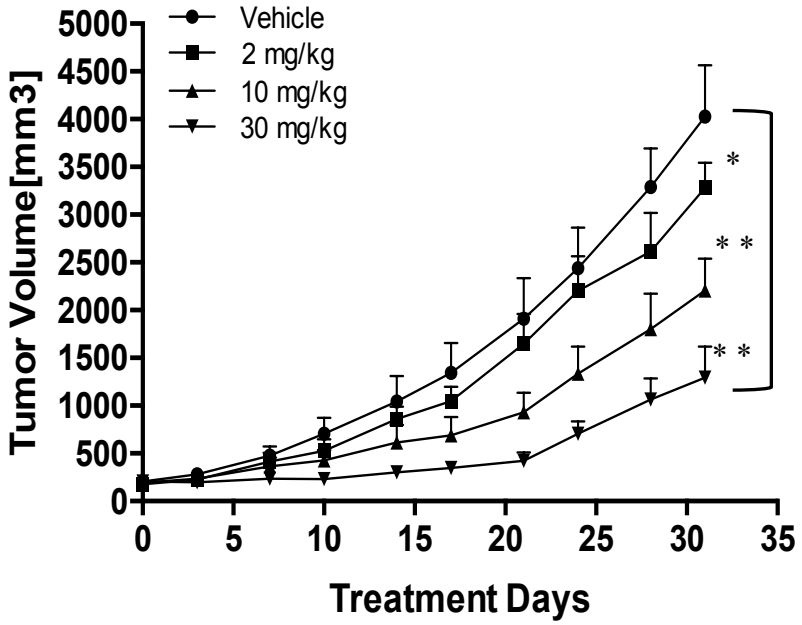


Figure 3. *MET*-addicted EBC-1 tumors display sensitivity to ABN401 *in vivo*

Nude mice with tumor xenografts established by subcutaneous injection of EBC-1 were treated 5 days continuously and 2 days off with vehicle (control) or ABN401 (2,10 or 30 mg/kg) for 3 weeks. Tumor volume was determined twice a week. Data shown are mean ± SE for 5 mice/group. *$p < 0.05$ for ABN401 (2 mg/kg), **$p < 0.001$ ABN401 (10 and 30 mg/kg) at 30 days versus the corresponding control value.

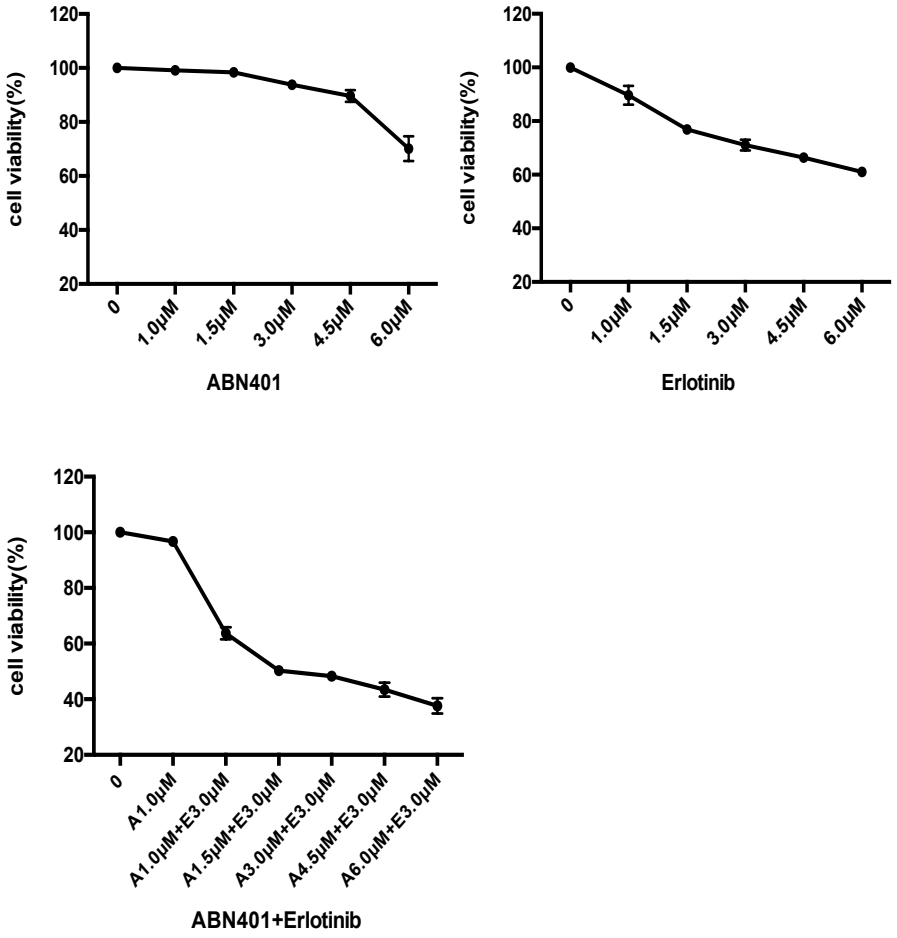


Figure 4. ABN401 plus Erlotinib strengthens inhibition of H1373 cell viability

H1373 cells viability assay performed after 72 hours of treatment with different concentration of ABN401, Erlotinib and a combination of both. Data shown are mean ± SD from triplicate.

Table 2. Chou Tatalay drug interaction analysis of ABN401 and Erlotinib

Cell viability %	ABN401 (μM)	Erlotinib (μM)	CI
64	1.0	3.0	0.69
50	1.5	3.0	0.34
48	3.0	3.0	0.44
43	4.5	3.0	0.48
38	6.0	3.0	0.50

*Combination index (CI) <1 indicates synergism

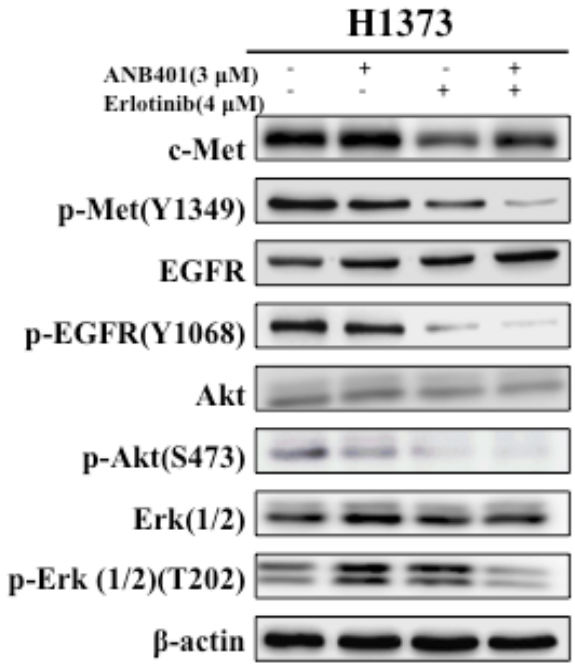


Figure 5. Inhibition by ABN401 and Erlotinib of MET, EGFR, and their downstream signaling

Western blot analyses of samples obtained from H1373 cells exposed to ABN401 (3 μM) or Erlotinib (4 μM) for 72 hours. Levels of total and phosphorylated forms of MET, EGFR, Akt, Erk were detected. Protein samples obtained from untreated and treated cells were separated by SDS-PAGE and immune-blotted with specific antibodies. β-actin served to ensure equal loading.

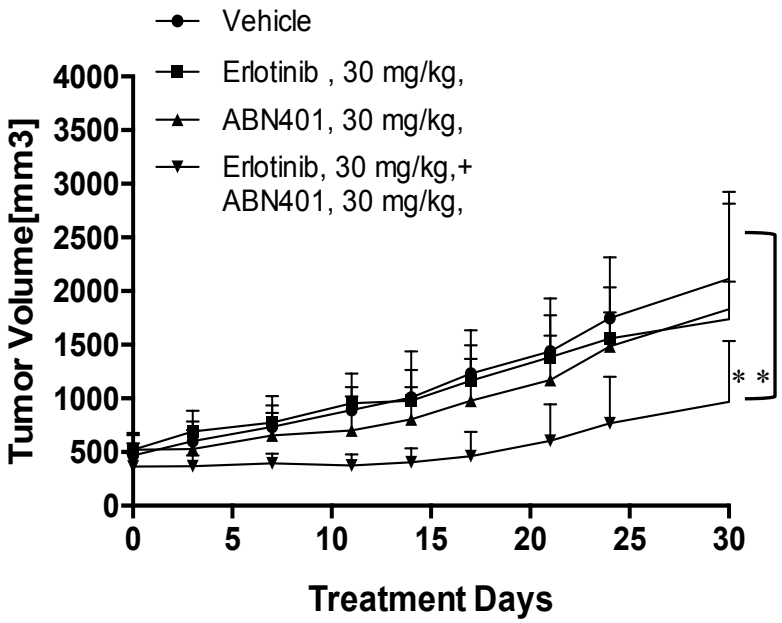
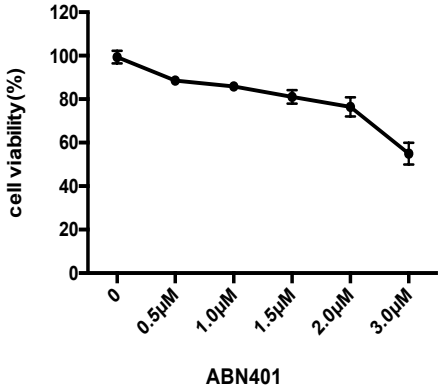
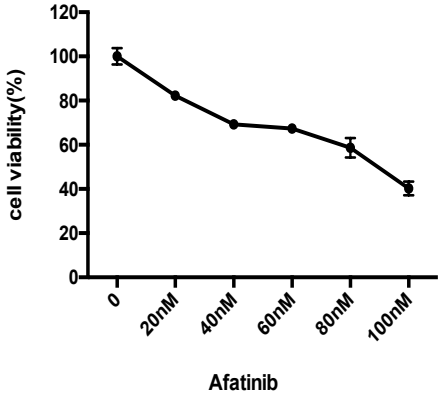
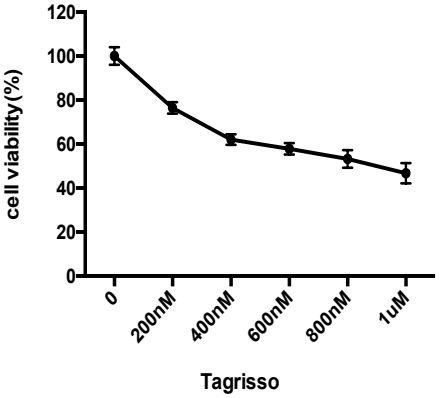


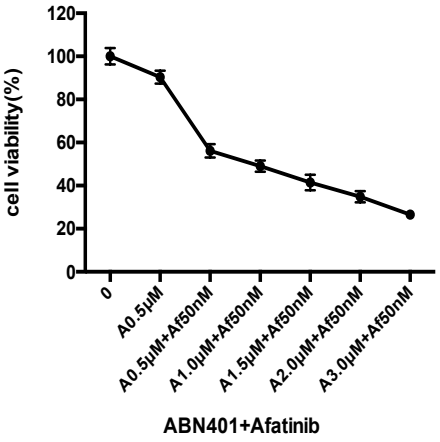
Figure 6. Combination of ABN401 and Erlotinib enhances inhibition of H1373 cell-derived xenograft tumors growth

Nude mice with tumor xenografts established by subcutaneous injection of H1373 were treated 5 days continuously and 2 days off with vehicle (control), ABN401 (30 mg/kg), Erlotinib (30 mg/kg), and ABN401 (30 mg/kg) plus Erlotinib (30 mg/kg) for 3 weeks. Tumor volume was determined twice a week. Data shown are mean ± SE for 5 mice/group. **p < 0.001 for ABN401 (30 mg/kg) plus Erlotinib (30 mg/kg) at 30 days versus the corresponding control value.


cell viability(%)
120
100
80
60
40
20
0
0
0.5μM
1.0μM
1.5μM
2.0μM
3.0μM
ABN401


cell viability(%)
120
100
80
60
40
20
0
0
20nM
40nM
60nM
80nM
100nM
Afatinib


cell viability(%)
120
100
80
60
40
20
0
0
200nM
400nM
600nM
800nM
1uM
Tagrisso


cell viability(%)
120
100
80
60
40
20
0
0
A0.5μM
A0.5μM+Af50nM
A1.0μM+Af50nM
A1.5μM+Af50nM
A2.0μM+Af50nM
A3.0μM+Af50nM
ABN401+Afatinib

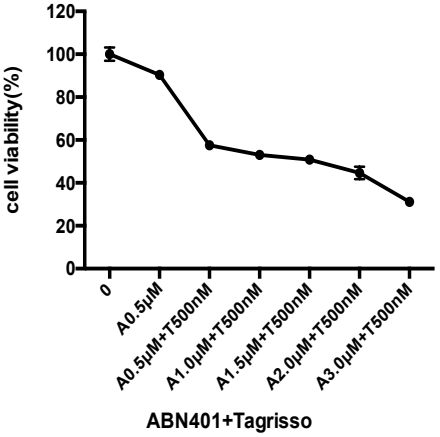

cell viability(%)
120
100
80
60
40
20
0
0
A0.5μM
A0.5μM+T500nM
A1.0μM+T500nM
A1.5μM+T500nM
A2.0μM+T500nM
A3.0μM+T500nM
ABN401+Tagrisso

Figure 7. Combined drug treatment of ABN401 and EGFR TKIs dramatically decreased cell viability in H820

H820 cells were treated with ABN401, Afatinib, Tagrisso or a combination of ABN401 with one of the other 2 drugs for 72 hours. Combined effects were measured using the WST assay. Data shown are mean ± SD from triplicate.

Table 3. Chou Tatalay drug interaction analysis of ABN401 and Afatinib

Cell viability %	ABN401 (μM)	Afatinib (nM)	CI
56	1.0	50	0.88
49	1.5	50	0.76
41	3.0	50	0.75
35	4.5	50	0.71
27	6.0	50	0.57

*Combination index (CI) <1 indicates synergism

Table 4. Chou Tatalay drug interaction analysis of ABN401 and Tagrisso

Cell viability %	ABN401 (μM)	Tagrisso (nM)	CI
58	1.0	500	0.59
53	1.5	500	0.58
51	3.0	500	0.81
45	4.5	500	0.83
31	6.0	500	0.57

*Combination index (CI) <1 indicates synergism

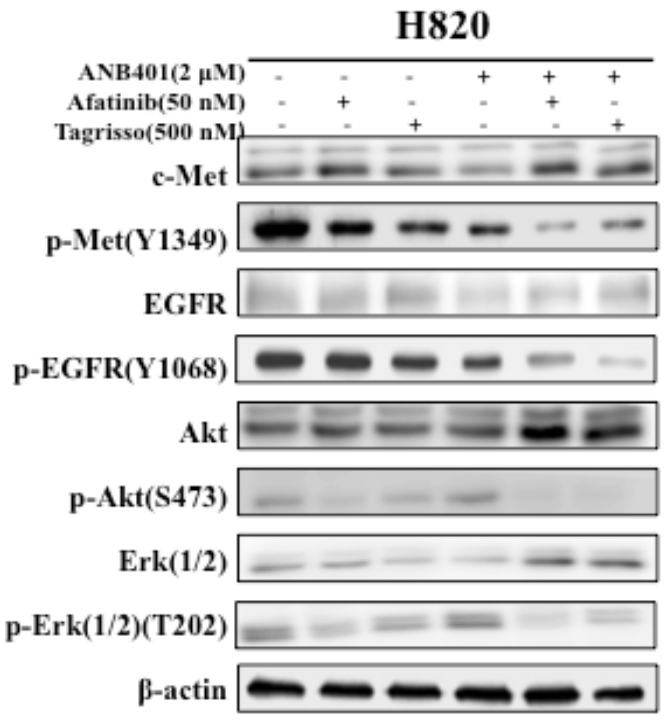


Figure 8. Combination of inhibiting MET and EGFR interrupts activation of cell survival signaling in H820

H820 cells were treated with ABN401 (2 μM), Afatinib (50 nM), Tagrisso (500 nM) or a combination of ABN401 with one of the other 2 drugs. After 72 hours, cells were harvested and subjected to Western blot.

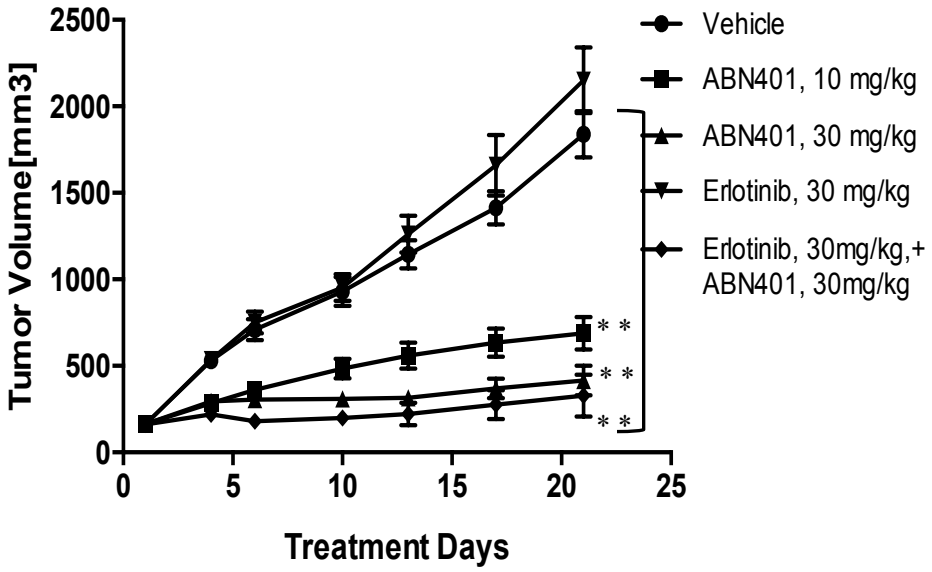


Figure 9. In *vivo* antitumor efficacy of ABN401 with or without Erlotinib on PDX NSCLC model

Human NSCLC patient-derived xenograft (PDX) tumor model was established for *In vivo* efficacy tests. Mice were treated 5 days continuously and 2 days off with vehicle (control), ABN401 (10 mg/kg), ABN401 (30 mg/kg), Erlotinib (30 mg/kg), and ABN401 (30mg/kg) plus Erlotinib (30 mg/kg) for 3 weeks. Tumor volume was determined twice a week. Data shown are mean ± SE for 8 mice/group. **$p < 0.001$ for ABN401 (10 and 30 mg/kg) and ABN401 (30 mg/kg) plus Erlotinib (30 mg/kg) at 21 days versus the corresponding control value.

DISCUSSION

For patients with advanced NSCLC, the lack of effective therapeutic options have prevents improving the poor prognosis and high mortality rate of the disease [18]. With the advanced understanding of lung cancer development and progression has led to new applications of targeted cancer therapy by inhibiting specific oncogenic drivers such as EGFR or MET. The MET pathway is frequently dysregulated in a wide variety of human cancers and may play key roles in cancer formation, progression, dissemination, and strong expression of MET, which is observed in 61% of NSCLCs [22]. With the possibility of MET as therapeutic target in various tumors, there have been ongoing researches in developing MET inhibitors, but there may be limitations during development such as unwanted off-target toxicities [23]. Here, it is described a newly developed c-Met kinase inhibitor, ABN401, which is currently being evaluated. ABN401 potently inhibited lung cancer cells proliferation and also blocks MET phosphorylation and activation of its key downstream molecular effectors especially in MET high-amplified cell lines. ABN401 also exhibited dose-dependent antitumor activity in xenograft tumor models driven by MET amplification. Collectively, the data suggested that ABN401 possesses potent both *in vitro* and *in vivo* biological and

pharmacologic activities and may provide a new therapeutic potential in lung cancer treatment.

However, targeting only MET has limitations due to the by-pass signaling by other types of receptor tyrosine kinase, such as EGFR. My results also indicated that cell lines in different cellular contexts such as H1373 and H820 showed less effect to ABN401. Therefore, I evaluated the benefit of inhibiting both MET and EGFR on NSCLC. *In vitro*, it appeared that combined drug treatment of ABN401 and EGFR TKIs dramatically decreased cell viability compared to single treatment. In addition, the activities of the drugs on signal transduction seem to be consistent with their activities on proliferation *in vitro*. This is not surprising, because both MET and EGFR can activate common downstream pathways, such as RAS-MAPK/ERK and PI3K-AKT, and through these pathways seem to trigger similar cellular activities including survival and proliferation [24,25]. So that, inhibiting either MET or EGFR alone would decrease the inhibition when both RTKs are functioning in the same cells. Similar results were found in *in vivo* tests, which can exactly reflect drug's efficacy in the human situation.

My research results strongly suggested that the combined inhibition of MET and EGFR can strengthen anticancer effects against NSCLCs in different cell environments and that it may be an effective therapeutic alternative that is superior to targeting an individual pathway. This is essential because it is known that human cancers are heterogeneous, but so far, there is no one-to-one treatment solution [26].

This also might explain why a lot of patients with NSCLCs carrying EGFR mutation do not respond well to EGFR targeted therapy: Activity of EGFR might not be the main driving force in such situation and alternative pathways like MET may also maintain tumor growth and survival [27].

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국문초록

Mesenchymal-epithelial transition factor(MET)은 hepatocyte growth factor(HGF)에 대해 강한 결합력을 가진 유일한 수용체로써 human carcinoma 에서 중요한 역할을 담당하여 치료대상의 중요 치료 타겟분자로 인식되고 있다. MET 과 epidermal growth factor receptor(EGFR)는 receptor tyrosine kinases 로, 상호간의 cross-talk 를 통하여 non-small cell lung cancer(NSCLC)를 발달, 진행시키는데, 본 연구에서는 ABN401 이라는 새로운 MET kinase inhibitor 만을 이용한 single treatment, 그리고 ABN401 과 EGFR inhibitor 인 Erlotinib, Afatinib, Tagrisso 를 각각 동시에 처리한 combination therapy 에 대한 전임상 실험을 수행하였다.실험결과, ABN401 이 MET 의 인산화, c-MET 의 key downstream effector 들의 활성화에 강한 inhibitor 로 작용하였고, MET amplified lung cancer cell lines 에서 그 효과가 가장 큰 것으로 나타났다. 뿐만 아니라, MET amplified mouse tumor model 에서도 약물용량 증가에 따른 종양성장 억제 효과를 확인 할 수 있었다. 나아가, EGFR 에 의해 증가된 intracellular signaling cascade 로 인한 MET 의 활성화, 신호전달에 대한 추가 연구를 수행하였고, In vivo, In vitro 모두에서 MET inhibitor 만을 이용한 single treatment 보다 MET inhibitor 와 EGFR inhibitor 를 동시에 처리한 combined treatment 그룹의 종양 성장 억제가 더 효과적임을 확인 할 수 있었다.본 연구는 ABN401 이 MET 의 강력하고 선택적인 inhibitor 로 lung

cancer 치료에 잠재력 있는 후보 물질임 시사한다. ABN401 의 single treatment 에서 나아가 MET inhibitor 와 EGFR inhibitor 를 모두 사용하는 combinational therapies 의 개발은 NSCLC 환자의 치료 효과를 크게 증대 시킬것이다.

주요어:MET amplification, EGFR-therapy, combination therapy, NSCLC
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