OTHERS

Phase I study of combined therapy with vorinostat and gefitinib to treat *BIM* deletion polymorphism-associated resistance in *EGFR*-mutant lung cancer (VICTROY-J) : a study protocol

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Abstract : The *BIM* deletion polymorphism is reported to be associated with poor outcomes of epidermal growth factor receptor (EGFR)-mutant non-small cell lung cancer (NSCLC) treated with EGFR-TKIs, including gefitinib. We have shown that a histone deacetylase inhibitor, vorinostat, can epigenetically restore BIM function and apoptosis sensitivity to EGFR-TKIs in *EGFR*-mutant NSCLC cells with *BIM* deletion polymorphisms. The purpose of this study is to determine the feasibility of combined treatment of vorinostat with gefitinib in *BIM* deletion polymorphism positive EGFR-mutant NSCLC patients. *BIM* deletion polymorphism positive *EGFR*-mutant NSCLC patients treated with at least one EGFR-TKI and one regimen of chemotherapy are being recruited to this study. Vorinostat (200-400 mg) will be administered orally once daily on days 1-7, and gefitinib 250 mg orally once daily on days 1-14. With a fixed dose of gefitinib, the dose of vorinostat will be escalated following a conventional 3+3 design. The primary endpoint is to define the maximum tolerated dose (MTD) of vorinostat combined with 250 mg of gefitinib. This is the first phase I study of combined therapy with vorinostat and gefitinib for NSCLC patients double selected for an *EGFR* mutation and *BIM* deletion polymorphism. J. Med. Invest. 64 : 321-325, August, 2017

Keywords **:** *EGFR mutation, BIM polymorphism, gefitinib, vorinostat, non-small cell lung cancer*

INTRODUCTION

The majority of patients with non-small cell lung cancer (NSCLC) with epidermal growth factor receptor (*EGFR*) activating mutations, such as exon 19 deletion and L858R point mutation, show marked responses to EGFR tyrosine kinase inhibitors (EGFR-TKIs), such as gefitinib, erlotinib, and afatinib (1-4). However, 20-30% of patients with *EGFR*-activating mutations show intrinsic resistance to EGFR-TKIs. Molecular mechanisms of the intrinsic resistance are not fully understood (5).

BIM, also called Bcl-2-like protein 11, is a pro-apoptotic molecule that belongs to the Bcl-2 family. BIM upregulation is essential for the induction of apoptosis in lung cancer cells with *EGFR* mutations treated with first-generation EGFR-TKIs, and a low BIM protein level is associated with resistance to EGFR-TKIs (6, 7). Recently, an East Asian-specific 2,903 bp deletion polymorphism in the *BIM* gene was discovered, whose incidence was around 13% and 0.5% for heterozygous and homozygous carriers, respectively (8). Importantly, the *BIM* deletion polymorphism results in the preferential splicing of exon 3 over the BH3-encoding exon 4 in the *BIM* pre-mRNA, and leads to the production of inactive BIM isoforms lacking the BH3 domain. This in turn reduces expression of pro-apoptotic BIM protein isoforms in *EGFR*-mutant lung cancer cell lines following TKI exposure and is sufficient to confer TKI resistance (8). Since its initial discovery, several meta-analyses have reported an association between *BIM* deletion polymorphism and shorter progression-free survival (PFS) of patients with NSCLC harboring *EGFR* mutations, who received gefitinib or erlotinib treatment (9-13).

Vorinostat (suberoylanilide hydroxamic acid [SAHA]), has been approved in 20 countries to date including Japan for cutaneous T-cell lymphoma as monotherapy, is a small-molecule inhibitor of histone deacetylase (HDAC) that induces cell differentiation, cell cycle arrest, and apoptosis in several types of tumor cell lines (14). We previously reported that the combined use of vorinostat and gefitinib was able to preferentially upregulate the expression of pro-apoptotic BIM isoforms in *EGFR*-mutant NSCLC cell lines with the *BIM* deletion polymorphism, and overcome EGFR-TKI resistance *in vitro* and *in vivo* (15). Two clinical trials, a phase I/II study combining gefitinib and vorinostat in patients with advanced NSCLC regardless of presence/absence of *EGFR* mutation in Korea (16) and a phase I/II study combining erlotinib and vorinostat with advanced *EGFR*-mutant NSCLC patients after EGFR-TKI progression in Spain (17) have been performed. However, the combination treatment did not show significant efficacy in these patient population and novel biomarker is warranted. Therefore, based on our preclinical findings, we designed the present phase I study named VICTORY-J "Vorinostat-Iressa Combined Therapy on Resistance by BIM Polymorphysim in EGFR Mutant Lung Cancer" to evaluate the safety of combined therapy with vorinostat and gefitinib, and to

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determine the maximum tolerated dose (MTD) of vorinostat combined with a fixed dose of gefitinib for Japanese patients with *EGFR*-mutant NSCLC with a *BIM* deletion polymorphism.

METHODS AND DESIGN

Purpose

The primary objective is to determine the MTD of vorinostat combined with a fixed dose of gefitinib for patients with *EGFR*-mutant NSCLC with a *BIM* deletion polymorphism. The secondary objective is to evaluate the safety and efficacy of the combined therapy with vorinostat and gefitinib in the early-phase trial setting.

Study design

This study is an open-label, multi-institutional phase I dose-escalation study of participating institutions, include 5 specialized centers in Japan as of November 2016. Participating institutions are listed in Appendix 1.

Three to six patients will be enrolled at each dose level of vorinostat. With a fixed dose of gefitinib, dose escalation of vorinostat following a conventional 3+3 design using an escalation scheme will be used (Figure 1). Initially, 3 patients are enrolled to level 1. If one or two patients experience DLT, 3 additional patients are enrolled to the level. If 3 of 6 patients experience DLT, the previous level is declared the MTD. If 2 or less of 6 patients experience DLT, dose escalation is permitted to continue. After the termination of protocol treatment, any treatment is allowed.

Ethical considerations and registration

This study was conducted in accordance with the International Committee for Harmonization Good Clinical Practice (ICH-GCP) guideline and the Declaration of Helsinki. The study protocol was approved by the institutional review boards of all participating institutions. Informed consent will be provided for all patients before registration. This study was registered with ClinicalTrials.gov (NCT02151721) and UMIN Clinical Trials Registry (UMIN 000015193).

Endpoint

The primary endpoint is MTD, which is defined as the highest dose level at which 2 or less of 6 patients experience a dose-limiting toxicity (DLT). Toxicities will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. DLT is defined as follows ; grade≥1 intestinal lung disease ; grade≥4 neutropenia lasting 5 days or more ; febrile neutropenia ; grade≥3 thrombopenia requiring platelet transfusion ; grade≥4thrombopenia ; any grade uncontrollable skin toxicity ; grade≥3 nonhematological toxicity. DLT will be evaluated during the first two cycles (14 days per cycle) of therapy.

The secondary endpoints are pharmacokinetics and pharmacodynamics of vorinostat and gefitinib, progression-free survival (PFS), overall survival (OS), response rate (RR), duration of response and complete response, disease control rate (DCR), and incidence of adverse events defined by Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

Eligibility criteria

Inclusion criteria

Prior to enrollment in the study, patients must fulfill all of the following criteria : histologically or cytologically diagnosed NSCLC (excluding squamous cell carcinoma) ; NSCLC of clinicopathologic stage IIIB or IV for which radical radiation therapy is impractical or there is a recurrence after surgery ; *EGFR* mutations (deletion of exon 19 and L858R mutation of exon 21) for which the clinical benefits of an EGFR-TKI (gefitinib or erlotinib) are recognized by testing methods that are listed by the national health insurance ; having a history of treatment with an EGFR-TKI (gefitinib or erlotinib) and a history of pathologic deterioration during treatment ; having a history of treatment with cytotoxic anticancer agents (not including pre- or postoperative chemotherapy in the previous 1 or

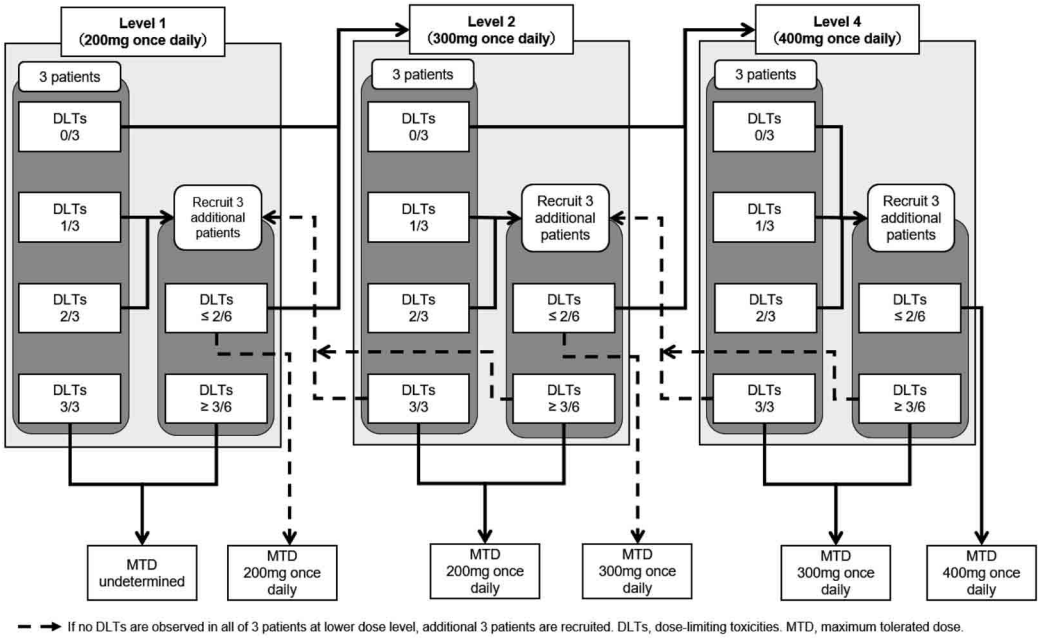

Figure 1. Study design.
Dashed arrow ; if no DLTs are observed in all of 3 patients at lower dose level, additional 3 patients are recruited. DLTs, dose-limiting toxicities. MTD, maximum tolerated dose.

more years from the day of final administration) ; confirmed *BIM* polymorphism by the PCR fragment analytical method and the sequence method at the central laboratory ; having a lesion measureable according to the RECIST guidelines version 1.1 ; confirmed progression of pathology at the site of irradiation after irradiation in a patient who only has an irradiated lesion ; age 20 years and older ; Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) 0 or 1 at the time of consent acquisition ; an estimated life expectancy of 12 or more weeks ; a patient whose acute toxicities in response to prior treatments have recovered to the baseline level in the most recent prior treatment, excepting adverse events considered not to be a safety concern at the discretion of the investigator ; acquisition of written informed consent to participate in the present study ; and adequate hematological, liver, renal and respiratory function within 14 days before entry as shown below.

i) Neutrophil count of≥1,500/μL.
ii) Platelet count of≥100,000/μL.
iii) Total bilirubin level of≤1.5-fold of the upper limit of reference value at each institution.
iv) Creatinine level of≤1.5 mg/dL.
v) PaO_2 of≥70 Torr or SpO_2 of≥94%.

Exclusion criteria

Patients will be excluded for any of the following reasons : they received the final administration of a cytotoxic anticancer agent within the past 4 weeks, they received the final administration of an EGFR-TKI within the past 7 days, or they received surgery or radiotherapy for a primary tumor or mediastinum within the past 6 months ; radiotherapy considered necessary ; having an interstitial lung disease or history thereof, radiation pneumonitis treated with corticosteroids or having a history thereof, a large volume of or uncontrollable pleural effusion, ascites, or pericardial effusion, or a serious infection and other serious complications ; detection of known resistance mutations of the *EGFR*, e.g., T790M ; suffering from a severe or poorly controlled systemic disease ; having an active or poorly controlled or symptomatic metastasis to the central nervous system, or an active double cancer ; verified hepatitis B virus antigen or C virus antibody positivity.

Patient registration and randomization

The investigators will send a patient registration form to the independent data center at Center for Advanced Medicine and Clinical Research, Nagoya University Hospital. Patient registration began on May 2014 and is set to continue until March 2017.

Treatment

Vorinostat (level 1 : 200 mg, level 2 : 300 mg, level 3 : 400 mg) is administered orally once daily on days 1-7, and gefitinib 250 mg orally once daily on days 1-14.

Follow-up

After completion of scheduled treatment, the patients will be followed up until death over a period of at least 1 year. All patients are required to undergo chest and abdominal computed tomography at 6-month intervals during the first 24 weeks and at 12-week intervals after this period. Brain magnetic resonance imaging or computed tomography is required following the same schedule but only for patients with brain metastasis at the time of enrollment (Table 1).

Sample size determination

Sample size was determined based on a conventional 3+3 phase I design for oncology drugs.

Statistical analysis

The population analyzed for the primary endpoint included the enrolled patients with complete safety data on DLT during the first two cycles.

RESULTS AND DISCUSSION

This is the first study to investigate the safety and preliminary efficacy of combined therapy with vorinostat and gefitinib for NSCLC patients with both *EGFR* mutations and a *BIM* deletion polymorphism. The double selection for *EGFR* mutations and a *BIM* deletion polymorphism is feasible. *EGFR* mutation analysis is covered by health insurance in Japan and is widely used in clinical practice for patients with NSCLC. The *BIM* deletion polymorphisms can be analyzed using DNA purified from mononuclear cells in 5 ml of peripheral blood within 14 days. We conducted a study, named PEOPLE-J, to screen for *BIM* deletion-positive individuals in the population of *EGFR*-mutant NSCLC patients. We identified more than 74 *BIM* deletion polymorphism-positive individuals among 500 *EGFR*-mutant NSCLC patients by 25th November 2016.

After determining the recommended dose of vorinostat combined

Table 1. Schedule of study assessments and evaluations.

		Screening	Treatment Cycle											Post-treatment
			1					2		3		4 and beyond	End of Treatment	Safety Follow-up
			day 1	day 2	day 3	day 4	day 8	day 1	day 8	day 1	day 8	day 1		30 days post-discontinued
Informed Consent	○													
Inclusion/Exclusion Criteria		○												
Blood test		○	○				○	○	○	○	○	○	○	
Chest X-P		○	○				○	○	○	○	○	○	○	
ECG		○	○				○	○					○	
Tumor Imaging		○	6-week (± 7 days) intervals during the first 24 weeks, and after this period, 12-week (± 14 days) intervals											
Blood for Pharmacokinetics			○	○	○	○								
Blood for Pharmacodynamics			○	○										
Review Adverse Events			←	—	—	—	—	—	—	—	—	—	→	

with gefitinib in Japanese *EGFR*-mutant NSCLC patients with *BIM* deletion polymorphism in this investigator-initiated trial, we would like to conduct phase II study in cooperation with pharmaceutical companies. If successful, this combined treatment with vorinostat and gefitinib may lead to substantial and important changes in the management of patients with *EGFR* mutant NSCLC with a *BIM* deletion polymorphism.

CONFLICT OF INTERESTS-DISCLOSURE

Yoshinori Hasegawa obtained speakers fees and research grant from AstraZeneka, Taiho, and MSD. Toshiaki Takahashi obtained speakers fees from AstraZeneca and Taiho and research grant from AstraZeneka, MSD, and Taiho. Nobuyuki Katakami obtained speakers fees and research grant from AstraZeneka and Taiho. Akira Inoue obtained speakers fees from AstraZeneca, Taiho and advisory fees from AstraZeneca and MSD. Seiji Yano obtained speakers fees and research grants from AstraZeneca and Taiho. The other authors have nothing to disclose.

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APPENDIX 1. Participating institutions

	Participating institution	Principal investigator
1.	Kanazawa University Hospital	Shinji Takeuchi
2.	Nagoya University Hospital	Yoshinori Hasegawa
3.	Institute of Biomedical Research and Innovation Hospital	Nobuyuki Katakami
4.	Tohoku University Hospital	Akira Inoue
5.	Shizuoka Cancer Center	Toshiaki Takahashi

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