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PHASE II STUDIES

Phase II evaluation of LY2603618, a first-generation CHK1 inhibitor, in combination with pemetrexed in patients with advanced or metastatic non-small cell lung cancer

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Summary *Introduction* LY2603618 is a selective inhibitor of checkpoint kinase 1 (CHK1) protein kinase, a key regulator of the DNA damage checkpoint, and is predicted to enhance the effects of antimetabolites, such as pemetrexed. This phase II trial assessed the overall response rate, safety, and pharmacokinetics (PK) of LY2603618 and pemetrexed in patients with non-small cell lung cancer (NSCLC). *Methods* In this open-label, single-arm trial, patients with advanced or metastatic NSCLC progressing after a prior first-line treatment regimen (not containing pemetrexed) and Eastern Cooperative Oncology Group performance status ≤2 received pemetrexed (500 mg/m^2, day 1) and LY2603618 (150 mg/m^2, day 2) every 21 days until disease progression. Safety was assessed using Common Terminology Criteria for Adverse Events v3.0. Serial blood samples were collected for PK analysis after LY2603618 and pemetrexed administration. Expression of p53, as measured by immunohistochemistry and genetic variant analysis, was assessed as a predictive biomarker of response. *Results* Fifty-five patients were enrolled in the study. No patients experienced a complete response; a partial re-

Key Message
This open-label, single-arm, phase II trial assessed the overall response rate, safety, and pharmacokinetics of CHK1 inhibitor LY2603618 and pemetrexed in patients with advanced or metastatic non-small cell lung cancer. No significant clinical activity of LY2603618 and pemetrexed combination therapy was observed, with results being comparable with historical pemetrexed single-agent data.

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sponse was observed in 5 patients (9.1 %; 90 % CI, 3.7–18.2) and stable disease in 20 patients (36.4 %). The median progression-free survival was 2.3 months (range, 0–27.1). Safety and PK of LY2603618 in combination with pemetrexed were favorable. No association between p53 status and response was observed. *Conclusions* There was no significant clinical activity of LY2603618 and pemetrexed combination therapy in patients with advanced NSCLC. The results were comparable with historical pemetrexed single-agent data, with similar safety and PK profiles being observed.

Keywords CHK1 protein kinase inhibitor · LY2603618 · Non-small cell lung cancer · p53 · Pharmacokinetics

Introduction

Non-small cell lung cancer (NSCLC), a genetically and histologically heterogeneous disease, is one of the leading causes of death from cancer worldwide [1]. Advances in treatment have improved outcomes [2]; however, there is a need for more treatment options. Personalized therapy based on histology, oncogenic targets, and predictive biomarkers is emerging as an important element for consideration in the development of new therapies.

Checkpoint kinase 1 (CHK1) is a protein kinase that plays a key role in the deoxyribonucleic acid (DNA) damage signal transduction pathway at checkpoints S and G2-M [3]. Preclinical studies suggest CHK1 inhibitors could serve as chemopotentiators when administered with DNA damaging agents, such as pemetrexed [4]. A phase I trial identified the recommended phase II dose of a CHK1 inhibitor, LY2603618 (150 mg/m^2), when combined with pemetrexed 500 mg/m^2 [5].

Loss of checkpoint regulation by CHK1 is compensated in normal cells by the checkpoint regulator p53, which controls the G1 checkpoint [6]. Dysfunctional TP53 is mutated in up to 46 % of all lung adenocarcinoma cases [7]. In tumor cells with dysfunctional p53, CHK1 is considered a primary mediator of DNA damage-dependent cell cycle arrest [8]. Therefore, pharmacological inhibition of CHK1 in combination with chemotherapy is thought to have potentially lethal effects in cells with p53 dysfunction [9].

This study evaluated the effect of LY2603618 combined with pemetrexed in patients with advanced NSCLC. It was hypothesized that the combination of LY2603618 and pemetrexed would yield higher response rates in patients because CHK1 inhibition would prevent prompt repair of pemetrexed-induced DNA damage.

Material and methods

Study design and drug administration

This was a single-arm, open-label, non-randomized, phase II study of LY2603618 and pemetrexed in patients with advanced or metastatic non-squamous NSCLC. The protocol was approved by an ethics committee and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. Eligible patients received pemetrexed 500 mg/m^2 (ALIMTA, Eli Lilly and Company, Indianapolis, IN) as a 10-min infusion on day 1 followed by LY2603618 150 mg/m^2 (Eli Lilly and Company, Indianapolis, IN) as a 1-h infusion approximately 24 h (day 2) after pemetrexed, repeating every 21 days. Folic acid, vitamin B_{12} supplementation, and dexamethasone were used in accordance with the pemetrexed label [10]. Patients were assessed for disease progression every 2 cycles of therapy (6 weeks). A biopsy of the primary tumor or a metastasis was required prior to treatment (during the baseline visit) and blood samples were collected during treatment.

The primary objective of this study was to determine the overall response rate (ORR) for patients receiving LY2603618 combined with pemetrexed. Key secondary objectives included characterizing safety and pharmacokinetic (PK) profiles for LY2603618/pemetrexed, exploring whether p53 function is a predictive biomarker, and assessing health-related quality of life (HRQOL) and symptom burden.

Patients

Adult patients, aged ≥18 years, with histological or cytological diagnosis of advanced or metastatic non-squamous NSCLC that progressed after first-line treatment with a platinum doublet chemotherapy regimen not containing pemetrexed were eligible. All patients provided written informed consent. Previous treatment with biological agents or radiation therapy (<25 % of bone marrow) was permitted. Patients had ≥1 measurable lesion according to Response Evaluation Criteria in Solid Tumors (version 1.1) [11] and a performance status of ≤2 on the Eastern Cooperative Oncology Group scale [12]. Adequate hematologic, renal, and hepatic function was required.

Exclusion criteria included whole pelvis radiation; treatment with an investigational drug/device within 28 days; concurrent enrollment in medical research not compatible with this study; a serious pre-existing/concomitant medical disorder; central nervous system metastases (unless successfully treated and off corticosteroids for ≥4 weeks prior to study); active infection; pregnant or lactating; positive results for human immunodeficiency virus, hepatitis B surface antigen, or hepatitis C antibodies; prior treatment with a CHK1 inhibitor or pemetrexed; concurrent non-steroidal anti-inflammatory

drug treatment; and clinically significant, uncontrollable third space fluid accumulation.

Pharmacokinetic analysis

PK analyses were performed on patients who received ≥1 dose of the study drug (LY2603618 or pemetrexed) and had sufficient samples to characterize each PK profile. Plasma samples were analyzed using validated liquid chromatography/mass spectrometry methods. Pemetrexed (day 1) and LY2603618 (day 2) PK parameters were derived from samples collected in cycles 1 and 2 immediately prior to the end of infusion and 1–2, 4–6, and 20–28 h post-infusion. PK parameters for LY2603618 were also derived from samples collected 167–191 h after infusion (day 8). Additional details regarding the pharmacokinetic analysis are reported in the supplemental methods.

Pemetrexed plasma concentration data were dose-normalized to a 500 mg/m^2 dose prior to analysis and similar PK parameters to those calculated for LY2603618 (with the exception of R_A and %AUC $_{(tlast-\infty)}$) were reported. For estimation of the pemetrexed half-life ($t_{1/2}$), plasma concentrations residing primarily within 1 to 25 h after initiation of the pemetrexed infusion were selected to maintain consistency with a previous pemetrexed PK analysis from the phase I study [5].

p53 biomarker analysis

For all patients, a fresh biopsy of the primary tumor or an accessible metastasis was required during the baseline visit. Formalin-fixed paraffin embedded (FFPE) preserved tissue was also requested, but not required for study entry. If analysis was performed on both the pre-treatment biopsy and archived tumor tissue, the data pertaining to the pre-treatment biopsy was used to determine functionality.

p53 immunohistochemistry

Automated immunohistochemistry (IHC) was performed for the detection of p53 in tumor tissue using the NeoMarkers Ab-5 (Thermo Scientific, Fremont, CA, USA) at a 1:400 dilution. The level of nuclear p53 positivity in the tumor cells and the percentage of tumor cells (indicated by a hematoxylin and eosin stain with nuclear positivity of p53) were assessed. If the p53 IHC percentage was <20 %, the p53 status was designated as functional, while ≥20 % was nonfunctional. If no tumor cells were observed, then the p53 status was designated as not determined.

TP53 genetic variant analysis

TP53 genetic variant analysis was performed in a tiered approach analyzing DNA from biopsy samples for p53 mutations in exons 5–9. If no non-synonymous mutations were observed, the sample was reanalyzed for mutations in exons 2, 3, 4, 10, and 11. TP53 exons 2–11 were amplified with specific primers designed to cover the coding sequence as well as splice junctions. Analysis was conducted using both SURVEYOR® Nuclease Digestion coupled to the Transgenomic WAVE® HS System (Omaha, NE) and bidirectional Sanger dideoxy sequencing. If ≥1 mutation was detected in exon 2–11, then p53 mutation status was designated as nonfunctional. If no mutation was detected, then the status was designated as functional.

Lung cancer symptom scale and average symptom burden index

The Lung Cancer Symptom scale (LCSS) patient scale [13] was administered to assess changes from baseline in symptom burden and HRQOL. The LCSS evaluable population consisted of all enrolled patients who had a baseline and ≥1 post-baseline measurement. Time to worsening of symptoms (TWS) was descriptively reported; a clinically significant change was defined as a 15-mm increase from baseline measured from the date of enrollment to the first date of a worsening.

The population was also evaluated for changes in the Average Symptom Burden Index (ASBI), with improvement/worsening based on trends seen in sets of consecutive ASBI assessments with respect to baseline. The ASBI was defined as the mean of all 6 symptom-specific items. Exploratory analyses evaluated the association between LCSS baseline values (ASBI, total LCSS score) and efficacy parameters. For the exploratory analyses, patients were identified as having low symptom burden (ASBI <25) or high symptom burden (ASBI ≥25). Twenty-five was the cut-off [14, 15] that aligned with the "mild" category of LCSS observer scale.

Statistical analyses

The primary objective was to estimate the ORR for patients receiving LY2603618 and pemetrexed. Since this was a single-arm design, a multicenter phase III trial evaluating pemetrexed versus docetaxel as a second-line therapy in patients with advanced or metastatic NSCLC was used as the reference study [16], where a response rate of 11.5 % was observed for subjects on pemetrexed therapy with non-squamous histology [17]. With a sample size of 55 patients, if 11 patients responded (ORR = 20 %) then the 90 % confidence interval (CI) for ORR was (11.6 %, 30.9 %). Note that

the lower bound of the 90 % CI is above 11.5 %, indicating that an ORR of at least 20 % (of the 55 subjects) implied efficacy. If fewer than 11 patients out of 55 responded, then it was concluded that pemetrexed + LY2603618 was not efficacious over pemetrexed alone.

Secondary efficacy endpoints were clinical benefit rate (CBR; complete response [CR] + partial response [PR] + stable disease [SD]), progression-free survival (PFS), and duration of response. Median PFS and median duration of response (including the 90 % confidence interval) were estimated using a Kaplan-Meier method.

For biomarker analysis, blood samples were collected at 4 time points (baseline, pre-dose day 1 of cycles 1, 2, and 3) to measure circulating tumor cells (CTCs), and on days 1–8 of cycle 1 and on day 1 of subsequent cycles for circulating DNA and cytokeratin 18 (CK18). Refer to the supplemental methods for more details on these analyses.

Adverse event (AE) terms and severity grades were assigned by the investigator using Common Terminology Criteria for Adverse Events version 3.0.

Results

Patient characteristics

Baseline patient and disease characteristics are summarized in Table 1. Of the 62 patients who entered the study, 55 patients received ≥1 dose of study drug. Seven patients failed study screening and did not receive any treatment. Patients had a median age of 62 years and were predominately male (61.8 %), with a pathological diagnosis of adenocarcinoma (87.3 %) and stage IV disease (87.3 %). All patients had received ≥1 prior systemic therapy. At the time of data cutoff (30 July 2012), 45 patients (81.8 %) had discontinued treatment due to disease progression. Other reasons for discontinuation included AEs (10.9 %), death (1.8 %), or withdrawal due to patient (1.8 %) or sponsor (1.8 %) decision. One patient with a PR continued on treatment until October 16, 2014, when he discontinued due to progressive disease after 4.5 years on therapy.

Efficacy

Of the 55 enrolled patients, 49 were evaluable for best overall response (Table 2). Six patients did not have a post-treatment radiological response assessment due to clinical progression ($n = 3$), discontinuation due to an AE ($n = 2$), or death ($n = 1$). All 55 patients were included in the overall analysis. No patients experienced a CR. A PR was observed in 5 patients (9.1 %) and SD in 20 patients (36.4 %). Twenty-four patients (43.6 %) had progressive disease (PD; Table 2). The clinical benefit rate was 45.5 %.

The median PFS was 2.3 months (range as of data cutoff date, 0–27.1). Forty-six patients experienced PD or died. Of the 9 censored patients, 6 had no known disease progression and 3 started another therapy.

Of the 5 patients with PR, 3 patients either progressed or died and 2 patients were censored, both of whom had no disease progression. The median duration of response was 8.7 months (range, 4.5–23.0). Of the 20 patients with SD, 17 patients either progressed or died and 3 were censored. For patients with SD, the median duration of SD was 4.2 months (range, 1.4–17.4).

Table 1 Baseline patient and disease characteristics

Characteristic	LY2603618 150 mg/m^2 + Pemetrexed 500 mg/m^2 ($N = 55$)
Sex, n (%)	
Male	34 (61.8)
Female	21 (38.2)
Age (years)	
Median	62.0
Range	38–78
Race, n (%)	
White	33 (60.0)
Black or African American	1 (1.8)
Asian	21 (38.2)
ECOG Performance Status, n (%)[a]	
0	23 (41.8)
1	30 (54.5)
2	1 (1.8)
Pathological Diagnosis, n (%)	
Adenocarcinoma	48 (87.3)
Non-small cell lung cancer, NOS	4 (7.3)
Large cell carcinoma	2 (3.6)
Squamous cell	1 (1.8)
Disease Stage, n (%)	
Stage III	7 (12.7)
Stage IV	48 (87.3)
Prior Anti-cancer Therapies, n (%)	
At least 1 prior systemic therapy[b]	55 (100.0)
At least 1 prior radiotherapy	23 (41.8)
At least 1 prior surgery	11 (20.0)

ECOG Eastern Cooperative Oncology Group, *NOS* not otherwise specified

[a] Baseline ECOG PS was not recorded for 1 patient

[b] Seven patients within this group had received 2 prior treatment regimens

Table 2 Best overall response (intent-to-treat population)

Primary efficacy measure	LY2603618 150 mg/m^2 + Pemetrexed 500 mg/m^2 (N = 55)
Best Overall Response, n (%) [90 % CI])	
Complete response (CR)	0
Partial response (PR)	5 (9.1) [3.7, 18.2])
Stable disease (SD)	20 (36.4) [25.6, 48.3])
Progressive disease	24 (43.6) [32.2, 55.6])
Not evaluable	3 (5.5) [1.5; 13.5])
Missing	3 (5.5)
Overall Response Rate (CR + PR), n (%)	5 (9.1) [3.7, 18.2])
Clinical Benefit Rate (CR + PR + SD), n (%)	25 (45.5) [33.9, 57.4])

CI Confidence interval

Safety

The incidence of treatment-emergent adverse events (TEAEs) occurring in ≥10 % of the safety population is shown in Table 3. The most common TEAEs related to study treatment were decreased neutrophils/granulocytes (27.3 %), nausea (21.8 %), decreased hemoglobin (16.4 %), fatigue (12.7 %), and vomiting (12.7 %). The most frequently reported Grade 3/4 TEAE was decreased neutrophils/granulocytes (21.8 %). Six patients (10.9 %) discontinued treatment due to AEs, with 4 events (convulsion, venous injury, dermatitis [cutaneous inflammation of leg tissue], and thrombocytopenia) being related to the study drug.

Seven patients (12.7 %) had ≥1 serious adverse event (SAE) assessed as possibly related to the study drug, including 2 events of decreased platelets and 1 event each of dermatitis, diarrhea, febrile neutropenia, sepsis, seizure, decreased neutrophils/granulocytes, and pulmonary embolism. One patient died of infectious colitis (not related to study drug treatment) after receiving 2 cycles of treatment. A total of 2 patients had 1 dose reduction of LY2603618 due to 1 case each of neutropenia and sepsis. Three patients had 1 dose reduction of pemetrexed due to 1 case each of fatigue, neutropenia, and sepsis.

Clinical pharmacokinetics of LY2603618 and pemetrexed

The PK profile of LY2603618 displayed a multi-exponential decline in plasma concentrations, consistent intra-patient PK behavior, and a relatively minor amount of intercycle accumulation of LY2603618 between cycle 1 and cycle 2 (Fig. 1a; Table 4). As demonstrated by the percent coefficient of variation (CV %) (Table 4), there appears to be a relatively moderate-to-large degree of between (inter-) patient LY2603618 PK variability. However, the PK parameters calculated after administration of LY2603618 150 mg/m^2 on day 2 of cycles 1 and 2 were consistent with data reported from a previous study that investigated pemetrexed and LY2603618 at the same dose levels [5].

The LY2603618 PK parameter target values of $AUC_{(0-\infty)} \geq 21{,}000$ ng h/mL and $C_{max} \geq 2000$ ng/mL, which were defined prior to the start of the study and correlated with the maximal pharmacodynamic effect observed in non-clinical xenograft models, were exceeded based on the geometric mean values for each PK parameter at 150 mg/m^2 (Fig. 1b). The geometric mean LY2603618 $t_{1/2}$ of 14.4 and 13.2 h in cycles 1 and 2, respectively, are consistent with a duration suitable for minimizing intercycle accumulation and for achieving the desired LY2603618 systemic exposure (Table 4). Overall, 9 patients (22 %) did not achieve at least 1 PK parameter target (AUC or C_{max} as defined above) for maximum pharmacodynamic effect on cycle 1, day 2 and 10 patients (21 %) did not achieve this on cycle 2, day 2. All 5 patients who achieved a PR exceeded at least 1 of the PK parameter targets (i.e., C_{max} and/or $AUC_{(0-\infty)}$).

Dose-normalized plasma pemetrexed concentration-versus-time profiles (Fig. 1c) and associated PK parameters (Table 4) were consistent between cycles 1 and 2 and similar to a previous study [5] with pemetrexed/LY2603618 and to those reported in the ALIMTA package insert [10], indicating the pemetrexed PK profile was unaffected by the administration of LY2603618 in this study.

p53 biomarker analysis

The functionality of p53 was assessed by both TP53 somatic mutational screening and IHC for p53 protein expression. A total of 40 samples were analyzed. Mutation results for 5 samples were not evaluable due to insufficient tumor content. A total of 35 samples had somatic mutation results in TP53 exons 2–11. Nineteen tumor tissue samples (47.5 %) had no TP53 somatic mutations identified and were designated as having a functional p53 pathway. The prevalence of TP53 mutations was 40.0 % (16/40 samples); 75 % of the samples with mutations (12/16 samples) contained mutations in the DNA binding domain (Fig. 2a). Two samples were found to have the same mutation in exon 5 (c.CGC > CTC; p. R158L), while the remainder of the mutations identified were unique to each sample.

A total of 40 samples were analyzed for p53 expression using IHC (Fig. 2b). Thirteen samples (32.5 %) had functional p53 status (<20 % IHC staining), 22 (55.0 %) had nonfunctional status (≥20 % IHC staining), and 5 could not be determined (Fig. 2b).

The p53 biomarker combination results (functional status by somatic mutation screening and IHC) were used to determine an overall p53 functionality status. For the 35 samples with both mutation and IHC functionality data available, 19

Table 3 Treatment-emergent (all causality and related) adverse events in ≥10 % of safety population

	LY2603618 150 mg/m^2 + Pemetrexed 500 mg/m^2 (N = 55)			
	All	All Grade 3/4	Related	Related Grade 3/4
Patients With ≥1 TEAE, n (%)	54 (98.2)	32 (58.2)	41 (74.5)	18 (32.7)
Fatigue	19 (34.5)	1 (1.8)	7 (12.7)	0
Nausea	17 (30.9)	0	12 (21.8)	0
Decreased neutrophils/granulocytes	16 (29.1)	12 (21.8)	15 (27.3)	11 (20)
Decreased hemoglobin	12 (21.8)	1 (1.8)	9 (16.4)	1 (1.8)
Constipation	11 (20.0)	0	2 (3.6)	0
Vomiting	11 (20.0)	0	7 (12.7)	0
Dyspnea	10 (18.2)	0	1 (1.8)	0
Decreased leukocytes (total WBC)	8 (14.5)	7 (12.7)	6 (10.9)	6 (10.9)
Dermatology/skin – other[a]	8 (14.5)	1 (1.8)	4 (7.3)	0
Anorexia	8 (14.5)	0	3 (5.5)	0
Diarrhea	8 (14.5)	1 (1.8)	3 (5.5)	1 (1.8)
Increased ALT, SGPT	7 (12.7)	2 (3.6)	3 (5.5)	0
Cough	7 (12.7)	0	0	0
Increased AST, SGOT	6 (10.9)	1 (1.8)	3 (5.5)	0
Dizziness	6 (10.9)	0	2 (3.6)	0

ALT alanine transaminase, *AST* aspartate transaminase, *SGOT* serum glutamic oxaloacetic transaminase, *SGPT* serum glutamic pyruvic transaminase, *TEAE* treatment-emergent adverse event, *WBC* white blood cell

[a] Includes rash, dermatitis, pallor, or skin wounds/lacerations from accidents

had the same functional status for both methods and 16 samples had discordant p53 functional status. Of the 16 samples with a discordant p53 status, 11 had nonfunctional IHC and/or functional mutation p53 status and 5 had functional IHC with a somatic mutation. For the 5 patients with a PR, 1 patient had functional p53, 1 nonfunctional, and the functionality of 3 patients could not be determined (including the patient on study treatment for 4.5 years). No evidence of association between p53 status and ORR, CBR, PFS, or number of cycles administered was observed, regardless of the method used to determine p53 functionality.

Circulating biomarkers

Biomarker assessments compared the mean maximum change and mean maximum absolute change from baseline in CTCs, CK18 M30, CK18 M65, CK18 M30/M65 ratio, and circulating DNA levels between ORR and CBR categories. No statistically significant associations between categories were observed in these analyses, except in the case of the maximum absolute change from baseline in the number of circulating CTCs and its association with ORR (Supplemental Table 1). However, the statistical significance in this case should be interpreted with caution due to the high standard deviation of the measurements relative to its mean: CR/PR (mean = 26.0, standard deviation =31.3), non-CR/PR (mean = 225.8, standard deviation =299.4).

Patients with clinical benefit showed a decrease in CTCs, while those who progressed had increasing levels of CTCs. However, there were no statistically significant differences in CTCs, CK18, or circulating DNA and response or clinical benefit.

Lung cancer symptom scale and average symptom burden index

The overall completion rate of the LCSS was 78.1 %. Patients (n = 36) with 4 post-baseline assessments in which ASBI was defined were characterized as having a symptom burden that had improved (26 %), worsened (13 %), or remained stable (39 %). Change in ASBI was reported as unknown for 10 patients (22 %). There was marked heterogeneity in TWS over the course of the study in the ASBI, the 6 individual symptoms, and composite indices (symptom distress, activity level, and quality of life).

LCSS baseline values were further analyzed in an exploratory fashion with respect to tumor response and PFS. Patients with confirmed response or greater than or equal to the median PFS had lower mean ASBI and mean total LCSS score (and therefore less symptom burden) than patients with no response or less than the median PFS. Patients with low symptom burden (ASBI < 25) had markedly higher median PFS than those with high symptom burden (4.0 [90 % CI 2.7–4.2] vs. 1.5 [90 % CI 1.3–2.2]).

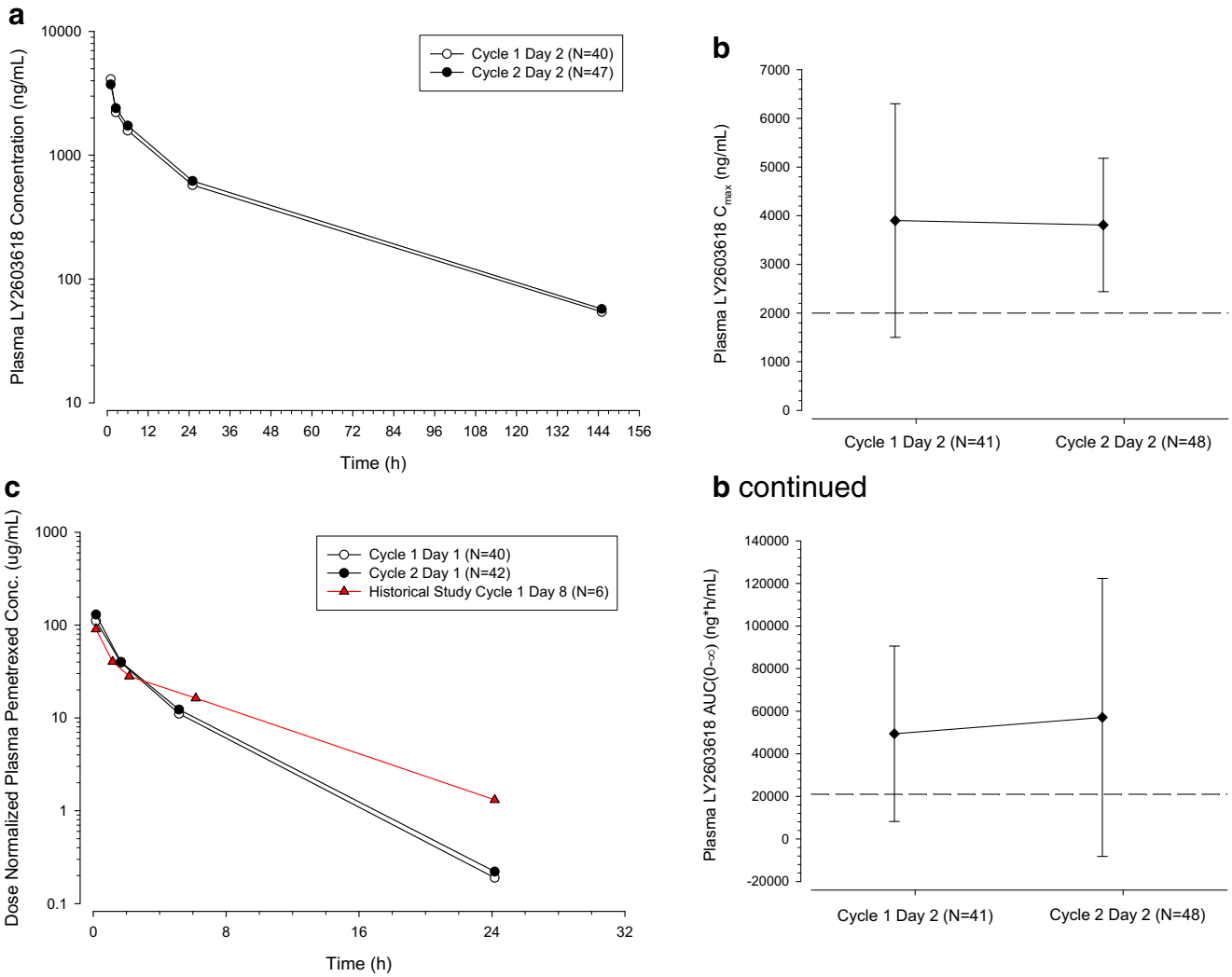

Fig. 1 **a** LY2603618 plasma pharmacokinetic profiles. The mean LY2603618 plasma concentration (logarithmic scale) versus time profiles following a 1-h infusion of 150 mg/m^2 LY2603618 in cycles 1 and 2. **b** Mean plasma LY2603618 Cmax and AUC(0-∞) values. Arithmetic mean (± standard deviation) LY2603618 C_{max} and $AUC_{(0-\infty)}$ values following a 1-h infusion of LY2603618 of cycle 1 and 2. Dashed lines represent C_{max} and $AUC_{(0-\infty)}$ values that correlate to the maximal pharmacodynamic effect observed in nonclinical xenograft models. **c** Dose-normalized pemetrexed plasma pharmacokinetic profiles. The mean dose-normalized pemetrexed plasma concentration versus time profiles following a 10-min infusion of 500 mg/m^2 pemetrexed in cycles 1 and 2 and the mean profile from an historical study [5] are graphically represented

Discussion

In this single-arm study, the addition of LY2603618 to standard second-line therapy with pemetrexed did not improve outcomes relative to historical controls [15]. Nonclinical studies have suggested that targeting cell cycle checkpoints via LY2603618 in combination with chemotherapy may be an effective therapeutic approach [4, 18]. Other CHK1 inhibitors, such as MK-8776 (SCH 900776), AZD7762, and GDC-0425, have shown responses in combination with gemcitabine and irinotecan [19–23]. Although these data support the rationale of combining CHK1 inhibitors with chemotherapy to enhance clinical outcomes, the primary objective was not met in this study.

Since this study was a single-arm study, a historical study that evaluated pemetrexed-only treatment was used as a reference [17]. It is noteworthy that changes in standard of care over time, inter-institution variability, and differences in prognostic factors may impact the validity of a historic control. In addition, endpoints estimated from the current study were estimates with associated variability and therefore any qualitative comparisons with historical pemetrexed data should be interpreted with caution.

The ORR observed in this study was 9.1 %, numerically smaller than the historic reference, and did not exceed the prespecified threshold. As a result, it is concluded that LY2603618 in combination with pemetrexed is not more effective than pemetrexed alone. However, the median duration

Table 4 Summary of LY2603618 and dose-normalized pemetrexed pharmacokinetic parameters

LY2603618 PK Parameters	Geometric Mean (CV%)		
	150 mg/m^2		
	Cycle 1 Day 2	Cycle 2 Day 2	
N	41	48	
C_{max} (ng/mL)	3430 (50)	3560 (40)[b]	
$AUC_{(0-\infty)}$ (ng·h/mL)	38,000 (85)	41,500 (88)	
$\%AUC_{(tlast-\infty)}$	2.93 (435)	2.33 (556)	
CL (L/h)	7.10 (84)	6.48 (87)	
V_{SS} (L)	134 (54)	114 (46)	
$t_{1/2}$ (h)	14.4 (86)	13.2 (98)	
R_A[a]	NC	1.15 (37)[c]	
Dose-Normalized Pemetrexed PK Parameters	**Geometric Mean (CV%)**		
	500 mg/m^2		Historical Data[d]
	Cycle 1 Day 1	Cycle 2 Day 1	Cycle 1 Day 8
N	40	43	6
C_{max} (μg/mL)	102 (50)	96.8 (42)	88.2 (27)
t_{max} (h)[e]	0.15 (0.15–1.50)	0.15 (0.13–0.37)	0.16 (0.15–0.47)
$AUC_{(0-\infty)}$ (μg·h/mL)	193 (31)	202 (33)	204 (74)
CL (L/h/m^2)	2.58 (31)	2.48 (33)	2.45 (74)
V_{SS} (L/m^2)	7.32 (33)	7.39 (28)	8.92 (20)
$t_{1/2}$ (h)	2.61 (18)	2.63 (19)	3.14 (38)

$AUC_{(0-\infty)}$ area under the plasma concentration-time curve from time 0 to infinity, *$\%AUC_{(tlast-\infty)}$* percent of $AUC_{(0-\infty)}$ extrapolated from $AUC_{(0-tlast)}$, *C_{max}* maximum plasma concentration, *CL* systemic clearance, *CV* coefficient of variation, *NC* not calculated, *PK* pharmacokinetic, *R_A* accumulation ratio, *$t_{1/2}$* terminal elimination half-life, *t_{max}* time of maximum observed plasma concentration, *V_{SS}* volume of distribution at steady state

[a] Intercycle accumulation ratio (Cycle 2, Day 2 $AUC_{(0-\infty)}$/Cycle 1, Day 2 $AUC_{(0-\infty)}$)

[b] One patient had an infusion of 3.18 h that was used to calculate the geometric mean and CV%

[c] n = 37

[d] Historical pemetrexed data are derived from a previous study that evaluated pemetrexed in combination with LY2603618 using the same dose and schedule of administration [5]

[e] Median (minimum–maximum)

of response to LY2603618 and pemetrexed was numerically higher (8.7 months) than that observed for pemetrexed (4.6 months) in the historical control study, regardless of histology. No clinical features were identified that were associated with duration of response.

It is hypothesized that LY2603618 may potentiate the chemotherapeutic effect to a greater degree in p53-nonfunctional tumors [9]. In this study, there was no association between p53 status and efficacy parameters. Although this study used both IHC and DNA mutations to assess p53 functionality, the assessment has limitations since standard IHC cut-points and mutation screening techniques have not been established for these evaluations. It is challenging to compare mutation rates due to differences in the specific exons screened, interpretation of functional mutations, patient populations, and stage of disease. However, the mutation frequency of p53 reported in the current study (40.0 %) is consistent with what has been previously reported [7, 24, 25]. It is also important to note that different mutations have different effects on p53 function. The nature of the mutation was taken into account in determining whether the sample was considered non-functional using available data from the COSMIC database. All non-functional alterations identified in this study were in the TP53 binding domain (amino acids 102–292), which are reported to lead to a loss of p53 function [26, 27].

Similarly, it is difficult to make direct comparisons between studies utilizing IHC due to variations in the p53 antibody clone utilized, the advanced NSCLC patient population analyzed, and the percent positive cut point for p53. This study utilized a conservative cut point (nonfunctional if ≥20 % staining). Using these criteria, 22/40 samples (55 %) had a p53 status that was nonfunctional. Even if a 10 % cutoff were used, 57.5 % of samples would have been nonfunctional. Two other studies that also used a similar antibody clone in a similar advanced NSCLC population reported that the percentage of p53 positive staining (>10 %) ranged from 30.1 % to 59 % [28, 29].

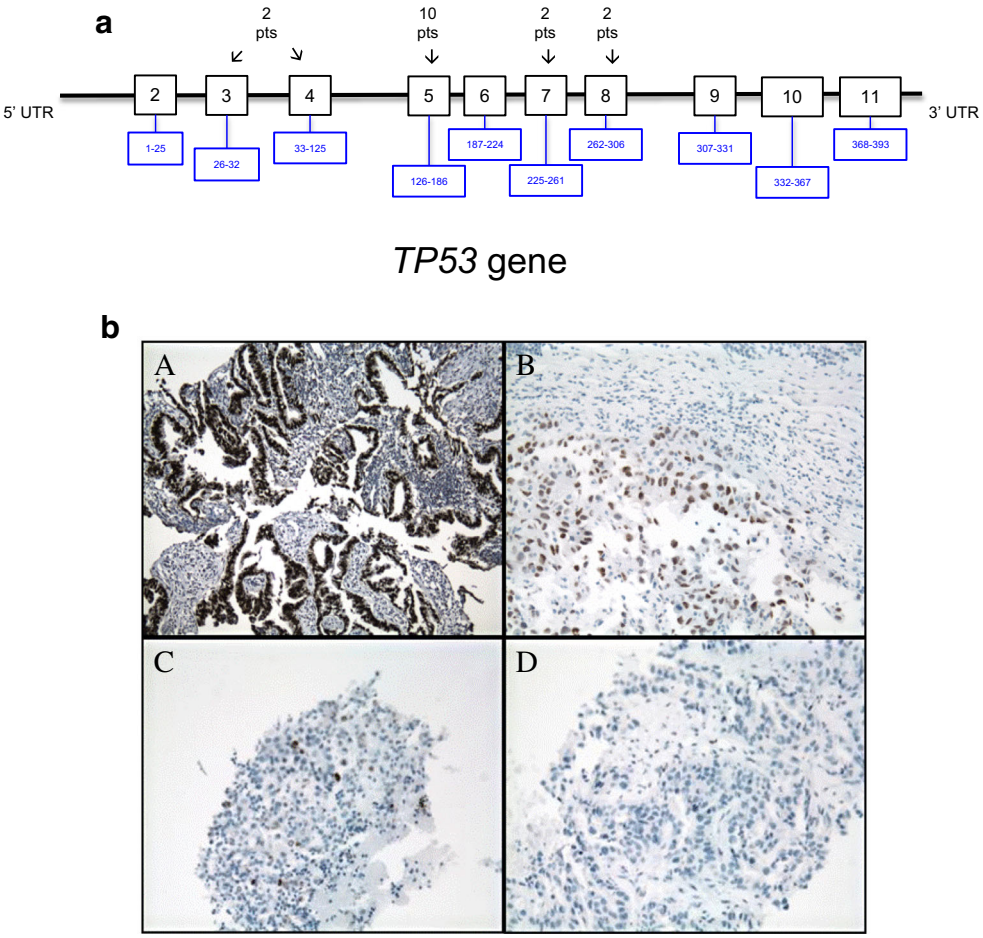

Fig. 2 **a** TP53 gene. An illustration of the TP53 gene and the number of patients with p53 mutations in select exons. b p53 immunohistochemistry. The variation in p53 expression among 4 different NSCLC patients' tumor tissue samples with approximately a 99 %, b 40 %, c 10 %, and **d** 0 % positive staining

Based on the 2 methods used to determine p53 functionality for the 35 available samples, 27/35 samples (77 %) had a nonfunctional p53 status and 8/35 samples (23 %) a functional p53 status. This nonfunctional p53 rate is higher than anticipated; other studies have reported ~50 % nonfunctional p53 rates in NSCLC patients. A possible explanation for this difference may be due to a higher sensitivity when combining both methods [26].

Although no evidence of association between p53 status and efficacy parameters was observed, due to the low ORR in this study, it was difficult to ascertain if p53 functionality using somatic mutation testing, IHC, or the combination of both was associated with response to LY2603618. Since over 60 % of patients had nonfunctional p53 by either mutation testing or IHC and the ORR was <10 %, there may be factors other than p53 that contributed to the outcome of this study.

The most common study drug-related TEAEs were decreased neutrophils/granulocytes, nausea, decreased hemoglobin, fatigue, and vomiting. The nature of these AEs is generally consistent with those reported for pemetrexed, suggesting that LY2603618 did not appreciably enhance toxicity when combined with pemetrexed. Grade 3/4 neutropenia was reported in 5.3 % of patients in the reference study and 29.1 % of patients in the current study. However, the rates of infection and febrile neutropenia were comparable with the historical study. All study drug-related SAEs, except for 1 seizure event attributed to LY2603618 treatment, were consistent with the known toxicity profile of pemetrexed.

The addition of LY2603618 to pemetrexed did not reduce the ability to administer full-dose pemetrexed. Only 3 patients in this study required dose reductions for pemetrexed. The overall dose intensity of pemetrexed in all patients was 96.7 % and the calculated pemetrexed PK parameters were similar to those reported for pemetrexed [10], suggesting that the addition of LY2603618 to pemetrexed did not influence PK parameters in a manner that may have influenced the study outcome.

The LY2603618 C_{max} and $AUC_{(0-\infty)}$ values that correlate to the maximal PD effect observed in nonclinical xenograft models [18] were exceeded based on the geometric mean values. Although all of the 5 patients who achieved a best overall response of PR exceeded at least 1 of the PK parameter targets (i.e., C_{max} and/or $AUC_{(0-\infty)}$), an additional exploratory post-hoc exposure-response statistical analysis revealed that there was no significant association between clinical responses and LY2603618 systemic exposure (Data on file).

It is not known why LY2603618 did not result in improved outcomes when combined with pemetrexed. Since there was not a direct PD biomarker, it is possible that in humans LY2603618 did not have sufficient potency or duration of CHK1 inhibition for a significant therapeutic effect. More potent, second-generation CHK1 inhibitors have entered clinical testing and will further test the hypothesis that CHK1 inhibitors may result in chemopotentiation.

In conclusion, the safety and PK profiles of the CHK1 inhibitor LY2603618 in combination with pemetrexed are consistent with the prior phase I study evaluating this combination. However, LY2603618 and pemetrexed did not appear to provide any additional clinical benefit to patients with advanced NSCLC.

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Compliance with ethical standards

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Conflicts of interest All remaining authors have declared no conflicts of interest.

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References

1. Siegel R, Ma J, Zou Z, Jemal A (2014) Cancer statistics, 2014. CA Cancer J Clin 64:9–29
2. Reck M, Heigener DF, Mok T, Soria JC, Rabe KF (2013) Management of non-small-cell lung cancer: recent developments. Lancet 382:709–719
3. Sanchez Y, Wong C, Thoma RS, Richman R, Wu Z, Piwnica-Worms H, Elledge SJ (1997) Conservation of the Chk1 checkpoint pathway in mammals: linkage of DNA damage to Cdk regulation through Cdc25. Science 277:1497–1501
4. Calvo E, Chen VJ, Marshall M, Ohnmacht U, Hynes SM, Kumm E, Diaz HB, Barnard D, Merzoug FF, Huber L, Kays L, Iversen P, Calles A, Voss B, Lin AB, Dickgreber N, Wehler T, Sebastian M (2014) Preclinical analyses and phase I evaluation of LY2603618 administered in combination with pemetrexed and cisplatin in patients with advanced cancer. Investig New Drugs 32:955–968
5. Weiss G, Donehower R, Iyengar T, Ramanathan RK, Lewandowski K, Westin E, Hurt K, Hynes SM, Anthony SP, McKane S (2013) Phase I dose-escalation study to examine the safety and tolerability of LY2603618, a checkpoint 1 kinase inhibitor, administered 1 day after pemetrexed 500 mg/m2 every 21 days in patients with cancer. Investig New Drugs 31:136–144
6. Gottifredi V, Karni-Schmidt O, Shieh SS, Prives C (2001) p53 down-regulates CHK1 through p21 and the retinoblastoma protein. Mol Cell Biol 21:1066–1076
7. The Cancer Genome Atlas Research Network (2014) Comprehensive molecular profiling of lung adenocarcinoma. Nature 511:543–550
8. Dai Y, Grant S (2010) New insights into checkpoint kinase 1 in the DNA damage response signaling network. Clin Cancer Res 16: 376–383
9. McNeely S, Beckmann R, Bence Lin AK (2014) CHEK again: revisiting the development of CHK1 inhibitors for cancer therapy. Pharmacol Ther 142:1–10
10. Alimta [package insert]. Indianapolis, IN: Eli Lilly and Company; 2012.
11. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J (2009) New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 45:228–247
12. Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, Carbone PP (1982) Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 5: 649–655
13. Hollen PJ, Gralla RJ, Kris MG (1995) Assessing quality of life in patients with lung cancer: a guide for clinicians. In: Gralla RJ, Moinpour CM (eds) *Assessing Quality of Life in Patients with Lung Cancer*: *A Guide for Clinicians*. NCM Publishers, New York, pp. 57–63
14. Wang P, Bowman L, Shen W, Winfree KB, Peterson P, John WJ (2012) The lung cancer symptom scale (LCSS) as a prognostic indicator of overall survival in malignant pleural mesothelioma (MPM) patients: Post hoc analysis of a phase III study. J Clin Oncol 30(suppl):7075 [abstract]
15. Obasaju C, Bowman L, Wang P, Shen W, Winfree KB, Smyth EN, Boye ME, John W, Brodowicz T, Belani CP (2013) Identifying the target NSCLC patient for maintenance therapy: an analysis from a placebo-controlled, phase III trial of maintenance pemetrexed (H3E-MC-JMEN). Ann Oncol 24:1534–1542
16. Hanna N, Shepherd FA, Fossella FV, Pereira JR, de Marinis F, von Pawel J, Gatzemeier U, Tsao TC, Pless M, Muller T, Lim HL, Desch C, Szondy K, Gervais R, Shaharyar, Manegold C, Paul S, Paoletti P, Einhorn L, Bunn PA Jr (2004) Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. J Clin Oncol 22: 1589–1597
17. Scagliotti G, Hanna N, Fossella F, Sugarman K, Blatter J, Peterson P, Simms L, Shepherd FA (2009) The differential efficacy of pemetrexed according to NSCLC histology: a review of two phase III studies. Oncologist 14:253–263
18. King C, Diaz H, Barnard D, Barda D, Clawson D, Blosser W, Cox K, Guo S, Marshall M (2014) Characterization and preclinical development of LY2603618: a selective and potent Chk1 inhibitor. Investig New Drugs 32:213–226

19. Daud AI, Ashworth MT, Strosberg J, Goldman JW, Mendelson D, Springett G, Venook AP, Loechner S, Rosen LS, Shanahan F, Parry D, Shumway S, Grabowsky JA, Freshwater T, Sorge C, Kang SP, Isaacs R (2015) Munster PN (2015) Phase I dose-escalation trial of checkpoint kinase 1 inhibitor MK-8776 as monotherapy and in combination with gemcitabine in patients with advanced solid tumors. J Clin Oncol 33:1060–1066
20. Infante JR, Hollebecque A, Postel-Vinay S, Bauer T, Blackwood B, Evangelista M, Mahrus S, Peale F, Lu X, Sahasranaman S, Zhu R, Chen Y, Ding X, Murray E, Schutzman J, Lauchle J, Soria J-C, LoRusso P (2015) Phase I study of GDC-0425, a checkpoint kinase 1 inhibitor, in combination with gemcitabine in patients with refractory solid tumors. Cancer Res 75(15 suppl):nr CT139 [abstract]
21. Sausville E, LoRusso P, Carducci M, Carter J, Quinn MF, Malburg L, Azad N, Cosgrove D, Knight R, Barker P, Zabludoff S, Agbo F, Oakes P, Senderowicz A (2014) Phase I dose-escalation study of AZD7762, a checkpoint kinase inhibitor, in combination with gemcitabine in US patients with advanced solid tumors. Cancer Chemother Pharmacol 73:539–549
22. Seto T, Esaki T, Hirai F, Arita S, Nosaki K, Makiyama A, Kometani T, Fujimoto C, Hamatake M, Takeoka H, Agbo F, Shi X (2013) Phase I, dose-escalation study of AZD7762 alone and in combination with gemcitabine in Japanese patients with advanced solid tumours. Cancer Chemother Pharmacol 72:619–627
23. Ho A, Bendell J, Cleary J, Schwartz GK, Burris HA, Oakes P, Agbo F, Barker PN, Senderowicz AM, Shapiro G (2011) Phase I, open-label, dose-escalation study of AZD7762 in combination with irinotecan (irino) in patients (pts) with advanced solid tumors. J Clin Oncol 29(suppl):3033 [abstract]
24. Gasco A, Molina-Vila M, Bertran-Alamillo J, Mayo C, Costa C, Capitan AG, Massuti B, Camps C, Costa EC, Ramirez SV, Martinez-Bueno A, Beniloch S, Capdevila L, Cros S, Porta R, Cardenal F, Bosch J, Sanchez JJ, Taron M, Rosell R (2012) Association of p53 mutations with progression-free survival (PFS) and overall survival (OS) in EGFR-mutated non-small cell lung cancer (NSCLC) patients (p) treated with erlotinib. J Clin Oncol 30(suppl 15):e18143 [asbtract]
25. Yamaguchi F, Kugawa S, Tateno H, Kokubu F, Fukuchi K (2012) Analysis of EGFR, KRAS and P53 mutations in lung cancer using cells in the curette lavage fluid obtained by bronchoscopy. Lung Cancer 78:201–206
26. Petitjean A, Mathe E, Kato S, Ishioka C, Tavtigian SV, Hainaut P, Olivier M (2007) Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. Hum Mutat 28:622–629
27. Robles AI, Linke SP, Harris CC (2002) The p53 network in lung carcinogenesis. Oncogene 21:6898–6907
28. Berghmans T, Mascaux C, Martin B, Ninane V, Sculier JP (2005) Prognostic role of p53 in stage III non-small cell lung cancer. Anticancer Res 25:2385–2389
29. Wang YC, Lin RK, Tan YH, Chen JT, Chen CY, Wang YC (2005) Wild-type p53 overexpression and its correlation with MDM2 and p14ARF alterations: an alternative pathway to non-small-cell lung cancer. J Clin Oncol 23:154–164