

**Brief Report: Evaluation of Antitumor Activity Using Change in Tumor Size of the  
Survivin Antisense Oligonucleotide LY2181308 in Combination with Docetaxel for Second-  
line Treatment of Patients with Non-small Cell Lung Cancer: A Randomized Open-label  
Phase II Study**

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**Running head:** Second-line LY2181308 and Docetaxel in Non-small Cell Lung Cancer

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## ABSTRACT

Chemoresistance is mediated, in part, by the inhibition of apoptosis in tumor cells. Survivin is an anti-apoptotic protein that blocks chemotherapy-induced apoptosis. To investigate whether blocking survivin expression would enhance docetaxel-induced apoptosis during second-line treatment of non-small cell lung cancer (NSCLC), we compared the antitumor activity of the survivin inhibitor LY2181308 plus docetaxel with docetaxel alone. We used change in tumor size (CTS) as a primary endpoint to assess its use in early decision-making for this and future studies of novel agents in NSCLC. Patients (N=162) eligible for second-line NSCLC treatment (stage IIIB/IV) with an Eastern Cooperative Oncology Group performance status of 0-1 were randomized 2:1 to receive LY2181308 (750 mg IV, weekly) and docetaxel (75 mg/m<sup>2</sup> IV, day 1) or docetaxel alone every 21 days. CTS from baseline to the end of cycle 2 was compared between the two treatment arms. The mean (SD) tumor size ratio for LY2181308/docetaxel and docetaxel was 1.05 (0.21) and 1.00 (0.15) ( $p = 0.200$ ), respectively. Because there was no significant difference between the two arms for progression-free survival (PFS) (2.83 months with LY2181308/docetaxel and 3.35 months with docetaxel [ $p = 0.191$ ]), both arms were combined to determine CTS. CTS reduction translated to longer PFS (PFS=4.63 months in patients with decreased CTS compared with 2.66 months in patients with increased CTS). The addition of LY2181308 to docetaxel in NSCLC showed no significant improvement in antitumor activity compared with docetaxel alone. CTS correlated with PFS, supporting its use in early decision-making in phase II studies.

**Key words:** Change in tumor size, NSCLC, docetaxel, survivin, LY2181308

## INTRODUCTION

Lung cancer is among the most common malignancies worldwide, and is the leading cause of cancer death in most countries.<sup>1</sup> Currently, patients with advanced or metastatic non-small cell lung cancer (NSCLC) who fail first-line treatment have several treatment options, including docetaxel, which has a median overall survival (OS) of 7.9 months and a median progression-free survival (PFS) of 2.9 months.<sup>2</sup> This limited response to second-line treatment results from chemoresistance associated with the inhibition of apoptosis.<sup>3</sup> One protein implicated in chemoresistance and failure to undergo apoptosis is the anti-apoptotic protein, survivin.<sup>4</sup>

In preclinical models, the survivin inhibitor, LY2181308, a second-generation antisense oligonucleotide, showed enhanced antitumor activity when combined with docetaxel.<sup>5</sup> These observations led to the clinical evaluation of a combination of LY2181308 and docetaxel in patients with NSCLC. It was hypothesized that blocking the expression of survivin with LY2181308 would restore default cell-death checkpoints and selectively eliminate cancer cells, leading to improved outcomes for NSCLC patients.<sup>6</sup>

To investigate the hypothesis that the combination of LY2181308 plus docetaxel is safe and potentially efficacious, we designed an open-label, randomized phase II study of second-line treatment in patients with NSCLC. Based on a previous publication,<sup>7</sup> the primary endpoint in this trial was change in tumor size (CTS) from baseline assessed at the end of cycle 2.

## PATIENTS AND METHODS

### *Patients*

Patients were at least 18 years of age and had a histological diagnosis of NSCLC (stage IIIB or IV at entry) that had progressed after one line of systemic chemotherapy. This study was

conducted in accordance with the ethical principles originating in the Declaration of Helsinki, good clinical practice, and all applicable laws and regulations. The ethical review board at each site approved the study, and all patients provided written informed consent before undergoing any study procedure.

### *Treatment and Study Design*

Patients were randomized in a 2:1 ratio to the experimental combination (LY2181308 and docetaxel arm) or standard-of-care treatment (docetaxel arm). Before cycle 1, patients in the LY2181308/docetaxel arm received two consecutive loading doses of 750 mg LY2181308 intravenously (IV) for 3 hours each.<sup>8</sup> A third loading dose was administered on day 1 of cycle 1, followed by weekly maintenance doses (on days 6 and 14) of 750 mg LY2181308. On day 1 of cycle 1, patients received 75 mg/m<sup>2</sup> docetaxel (administered over approximately 1 hour) 1 hour after LY2181308 infusion. Beginning with cycle 2 (and beyond), LY2181308 was administered on days 1, 8, and 15, with docetaxel 75 mg/m<sup>2</sup> on day 1, of each cycle until disease progression. The duration of each cycle was 21 days, with the first day of each cycle determined by administration of docetaxel (see Supplementary Fig. 1 for study diagram).

The primary objective was to evaluate the antitumor activity of LY2181308 in combination with docetaxel using CTS from baseline to the end of cycle 2 as an endpoint, as proposed by Karrison et al.<sup>9</sup> The approach of using tumor size measurements as a continuous rather than categorical variable for assessing antitumor activity preserves information at the patient level and increases statistical efficiency as suggested by Lavin et al.<sup>10</sup> Secondary objectives included a comparison of the following between treatment arms: PFS, OS, objective response rate (ORR), safety, pharmacokinetic profiles, plasma CK19 fragment (CYFRA21-1) and carcinoembryonic antigen (CEA).

### *Endpoint Assessments*

For the analysis of CTS, tumors were measured per Response Evaluation Criteria in Solid Tumors (RECIST) 1.1<sup>11</sup> using serial high-resolution computed tomography imaging. Scans of the chest/abdomen/pelvis were performed at baseline and every other cycle. All images were collected at 3-mm slices or with higher resolution, read by local investigators and by a central assessment (VirtualScopics; Rochester, NY). Safety was assessed every cycle by analyzing adverse events (AEs, graded per Common Terminology Criteria for Adverse Events [CTCAE] v4.0) and serious adverse events (SAEs). CYFRA21-1 and CEA were measured in plasma at baseline and before each cycle at a central laboratory (Quintiles).

### *Statistical Analyses*

Patients who received at least one dose of study drug were included in all statistical analyses. All tests of treatment effects were conducted at a two-sided alpha level of 0.05.

The analysis of the primary endpoint (CTS) was planned to occur after 120 patients received at least 2 cycles of therapy. With this sample size, the chances of detecting a difference in CTS at the 5% significance level were 100%, 99%, or 86% if the assumed differences in CTS were 60%, 50%, or as low as 40%, respectively, based on simulations. CTS, analyzed as the log of the ratios of tumor size at visit 2 to tumor size at baseline, follows a normal distribution and was compared between treatment arms using a t-test. The primary analyses were based on the measurements obtained from the central imaging assessment. Kaplan-Meier curves were produced for each time-to-event variable<sup>12</sup> and the differences between arms were assessed with the log-rank test. The effect of prognostic factors on PFS was assessed using a Cox proportional hazards model.<sup>13</sup>

## RESULTS

### *Patient Disposition*

The study was conducted from May 2010 to June 2012. A total of 207 patients entered the study, of which 120 were randomized to LY2181308/docetaxel and 60 to docetaxel (docetaxel monotherapy) (Supplementary Fig. 2). Patient demographics were similar between the two arms with respect to age, race, sex, and Eastern Cooperative Oncology Group performance status (Supplementary Table 1).

### *Change in Tumor Size*

Based on central imaging data, the mean (SD) tumor size ratio at cycle 2 to that at baseline was 1.05 (0.21) with LY2181308/docetaxel and 1.00 (0.15) with docetaxel ( $p = 0.200$ ). These data coincided with the investigator-assessed CTS evaluation (Table 1) (1.07 [0.28] with LY2181308/docetaxel versus 1.04 [0.28] with docetaxel;  $p = 0.666$ ). A waterfall plot for CTS was produced for the treatment groups based on the central imaging data (Supplementary Fig. 3). Tumor size diameter by visit and treatment is depicted in Supplementary Fig. 4.

### *Progression-free Survival*

The median PFS was 2.83 (95% CI, 1.84–3.65) months with LY2181308/docetaxel and 3.35 (95% CI, 2.69–4.57) months with docetaxel ( $p = 0.191$ ), as shown in Supplementary Table 2 and Fig. 1A.

As depicted in a forest plot (Fig. 2A), CTS (increase  $\nu$  decrease), age ( $<65 \nu \geq 65$ ), and lactate dehydrogenase (LDH) (high  $\nu$  low) were identified as statistically significant factors affecting PFS (Fig2B-D). For both arms combined, CTS reduction translated to longer PFS (PFS=4.63

months in patients with decreased CTS compared with 2.66 months in patients with increased CTS).

The relationship of PFS with detection of CYFRA21-1 and CEA ( $N=50$  and  $51$ , respectively)<sup>14</sup> showed that low baseline CYFRA21-1 levels were associated with a PFS of 5.62 months (95% CI, 2.66–5.91), whereas the median PFS for patients with a high CYFRA21-1 baseline value was 1.61 months (95% CI, 1.35–2.86).

### *Overall Survival*

The median OS (90% CI) was 7.9 (6.6–9.7) months with LY2181308/docetaxel and 8.8 (5.7–13.8) months with docetaxel ( $p = 0.481$ ) (Supplemental Table 2 and Fig. 1B).

### *Tumor Response*

Response rates are summarized in Supplemental Table 2. Partial response (PR) was observed in seven patients in the LY2181308/docetaxel arm and one patient in the docetaxel arm, yielding ORRs (95% CI) of 6.1% (1.7%–10.5%) and 2.1% (2.0%–6.1%), respectively ( $p = 0.438$ ).

### *Safety*

Ten (8.8%) patients in the LY2181308/docetaxel arm and 3 (6.3%) patients in the docetaxel arm discontinued due to SAEs considered possibly related to study drug. Patients in the LY2181308/docetaxel arm had a slightly higher incidence of grade 3/4 neutropenia, fatigue, anemia, and lung infection (Supplemental Table 3).

### *Pharmacokinetics*

Pharmacokinetics of LY2181308 alone, docetaxel alone, and docetaxel in combination with LY2181308 are provided as Supplementary Figs. 5–7 and were consistent with known profiles.



## **DISCUSSION**

Antitumor activity seen in preclinical models<sup>5</sup> did not translate to clinical benefit in the present randomized phase II study comparing LY2181308 and docetaxel with standard docetaxel in patients with NSCLC. There are several possible reasons for our findings. First, although the dose and schedule of LY2181308 used in this study were previously shown to reduce survivin,<sup>8</sup> tumor tissue was not obtained to confirm target inhibition. Secondly, this trial did not select patients on the basis of histology or biomarker or tumoral target expression, raising the possibility that benefit in a subset of patients was missed. Finally, we cannot exclude the possibility that there are other more dominant or common chemoresistant pathways in NSCLC in the second-line setting, rendering the role of survivin on apoptosis less critical.

Among the endpoints used in this study, CTS was chosen as a primary evaluation of antitumor activity because it can serve as an early evaluation of efficacy in phase II studies.<sup>9</sup> Although no statistically significant difference was observed between treatment arms, CTS did emerge as a reliable early endpoint in this trial across the entire study population, with a significant association between CTS and PFS. From a clinical trial design perspective, CTS appears to be an effective early marker of efficacy, with relatively short time intervals, that can aid in monitoring the efficacy of the treatment course, especially in early phase clinical trials.

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## FIGURE LEGENDS

**Figure 1. Kaplan-Meier graphs of PFS and OS.** (A) Kaplan-Meier graph for PFS, all treated patients; (B) Kaplan-Meier graph for OS, all treated patients. OS, overall survival; PFS, progression-free survival.

**Figure 2. Evaluation of influence factors on PFS.** (A) Influence of various baseline and prognostic factors on PFS, patients on therapy; (B) Kaplan-Meier graph for PFS by CTS at visit 2 split at median, patients on therapy; (C) Kaplan-Meier graph for PFS by age group, patients on therapy; (D) Kaplan-Meier graph for PFS by LDH split at median, patients on therapy. CTS, change in tumor size; ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase.

**Supplementary Figure 1. Study design diagram.** CTS, change in tumor size; N, population size; PFS, progression-free survival; PK, pharmacokinetics.

**Supplementary Figure 2. CONSORT diagram.** Of 207 patients entered, 27 patients were not enrolled. The remaining 180 patients were randomized into experimental ( $n=120$ ) and control ( $n=60$ ) arms. At least one dose of study drug was received by 114 patients in the experimental arm and 48 patients in the control arm.

**Supplementary Figure 3. Waterfall plot for CTS at visit 2 (Central Imaging Data, VirtualScopics).** Note: Sum of lesion diameters calculated per RECIST 1.1 guidelines.

**Supplementary Figure 4. Tumor size diameter by visit and treatment (Central Imaging Data, VirtualScopics).** Note: Sum of lesion diameters calculated per RECIST 1.1 guidelines. The box represents the median and the interquartile range and the diamond represents the mean.

Change in tumor size was measured as the log ratio of tumor size at visit 2 to tumor size at baseline (see content within highlighted red square).

**Supplementary Figure 5. Simulated (1000 Monte-Carlo simulations) plasma LY2181308**

**PK profiles.** PK model from study JACR after dosing on days 3 and 8 (*A*), and after the last dose (*B*) against observed PK data from this study (JACW). The dose is LY2181308 750 mg (3-hour infusion).

**Supplementary Figure 6. LY2181308 covariate model relationship and goodness of the fit plots.**

**Supplementary Figure 7. Docetaxel versus time profiles (mean) following docetaxel 75 mg/m<sup>2</sup> administration alone and in the presence of LY2181308.** Calculated using the interpretable individual profiles (that is, excluding the non-interpretable profile and outliers) and semi-log scale.