Inhibition of tumor growth and metastasis in non-small cell lung cancer by LY2801653, an inhibitor of several oncokinas, including MET
Corresponding author: Wenjuan Wu

SUPPLEMENTARY MATERIALS AND METHODS

Evaluation of LY2801653 antitumor effect in human lung cancer cell lines and patient-derived tumor (PDT) derived xenograft models

For xenograft models of NSCLC cell lines, logarithmically growing NCI-H441, A549, and H1975 tumor cells (over 95% viability) at the cell density of 5 x 10^6 cells 1:1 mixed with Matrigel (BD Biosciences, San Jose, CA) in 200 μL PBS were subcutaneously implanted onto female athymic nude mice (6-7 weeks old, obtained from Harlan laboratories, Indianapolis, IN) as previously described (1). When average tumor volume reached 100 mm^3, mice were randomized into various groups (n=7-10/group) and treated with LY2801653 (orally dosed) alone or in combination with gemcitabine (IP dosed), cisplatin (IV dosed) or erlotinib (orally dosed). Tumor volume= (π x length x width^2) / 6, where length represents the largest tumor diameter and width represents the perpendicular tumor diameter. The percent treatment/control (T/C%) values or percent regression (Regression %) values were calculated using the formula: T/C% = 100 × ΔT/ΔC if ΔT >0. Regression% = 100 × ΔT/T_initial if ΔT <0. T = mean tumor volume of the drug-treated group; ΔT = mean tumor volume of the drug-treated group on the study day of interest – mean tumor volume of the drug-treated group on initial day of dosing; T_initial = mean tumor volume of the drug-treated group on initial day of dosing; C = mean tumor volume of the control group; and ΔC = mean tumor volume of the control group on the study day of interest – mean tumor volume of the control group on initial day of dosing. The means are the least squares means (LS means) from the repeated measures ANOVA model described in the statistical analysis section of this supplemental material.
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For PDT xenograft models (LXFL-529, LXFL-430, LXFL-1176, LXFA-1647 and LXFA-526 derived by Oncotest GmbH, Germany), the tumors obtained from xenografts in serial passage in nude mice following their primary implantation were cut into small fragments (4-5 mm diameter) and subcutaneously implanted onto female NMRI nu/nu mice (Harlan, Netherlands) as previously described (2). When average tumor volume reached a predetermined size range, mice were randomized into various groups (n=7/group) and treated with LY2801653 (orally dosed) alone or in combination with erlotinib (orally dosed). Tumor volumes were measured bi-weekly and estimated by using the formula: Tumor volume= length x width²/2, where length represents the largest tumor diameter and width represents the perpendicular tumor diameter. Relative tumor volume was calculated by using the formula: Relative tumor volume = Tx (absolute tumor volume of the respective tumor on day x) x 100/T0 (absolute tumor volume of same tumor on day 0, when the treatment started). Mouse body weight was also measured bi-weekly. The percent treatment/control (T/C%) values or percent regression (Regression %) values were calculated using the formula above on summary statistics (medians instead of LS means).

Inhibition% was calculated as a relative level of tumor growth change for each treatment when compared to the vehicle group on the day of interest. A non-palpable or unmeasurable tumor was considered to be 100% inhibition.

**Histology and immunohistochemistry**

For human NSCLC patient tumor samples, two sets of samples were obtained: a tumor microarray (TMA) slide containing five-micron sections of 39 formalin-fixed human lung adenocarcinoma in 1.5-mm cores (Shanghai Biochips, China) and 13 frozen tumor samples (Indivumed, Germany). The frozen tumor sections were thawed and fixed in 10% neutral buffered formalin and routinely processed, embedded in paraffin, and microtomed at 4 microns.
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Sections were immunolabeled for MET (A2, a mouse anti-human MET monoclonal antibody, Eli Lilly and Company) using heat-induced antigen retrieval and standard immunohistochemical procedures and 3,3’-diaminobenzidine as chromagen. All immunohistochemically labeled slides were lightly counter-stained with hematoxylin. The immunohistochemical results were evaluated semi-quantitatively by the pathologist.

**Statistical analysis**

For the orthotopic tumor model, the data were analyzed using a one-way analysis of variance (ANOVA) model with Fisher’s least significant difference method for pair wise comparisons. The survival analysis was conducted using the Kaplan-Meier curve and the log rank test.

For human lung xenograft model of cancer cell lines, the statistical analysis was conducted on the log transformed tumor volumes. The log transformation has been shown to equalize variance across time and treatment groups for most tumor growth studies of this type. The log volume data were analyzed with a two-way repeated measures analysis of variance by time and treatment using the MIXED procedure in SAS software (Version 9.1.3, 9.2 or 9.3). The correlation model for the repeated measures is spatial power. Treated groups were compared to the control group at each time point. The MIXED procedure was also used separately for each treatment group to calculate LS means and standard errors at each time point. Both analyses account for both the autocorrelation within each animal and missing data. The means and standard errors were plotted for each treatment group versus time. In combination studies, further analysis was done to identify if the effect of the combination resulted in an additive or synergistic tumor growth inhibition. This additional analysis was conducted using SAS software (Version 9.1.3, 9.2 or 9.3) by testing for significance of a $2 \times 2$ interaction effect on log volume using the vehicle, each
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single agent and the combination of each single-agent groups. This analysis is referred to as synergy of effect (3). The study day for the data shown in Table S1 was chosen to be the final day of the study for which there were reliable data for all treatment groups.

For PDT xenograft studies except the LXFA-526 combination study, the nonparametric Kruskal-Wallis test and Dunn’s Multiple Comparison test were performed for the evaluation of the statistical significance of tumor inhibition. The data were analyzed for the LXFA-526 combination study by using the repeated measured ANOVA model described above.

References

