CDK4/6 Inhibitor (LY2835219) exhibits potent anti-tumor activity in human lung cancer cell lines with intact retinoblastoma

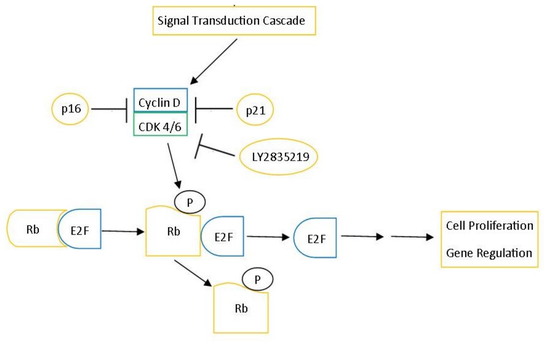
Jonathan W. Goldman, Edward B. Garon, Dylan Conklin, Deborah J.L. Wong, Brian R. Wolf, Danielle D. Silveira, Naeimeh Kamranpour, Sharon Pitts, Richard S. Finn

David Geffen School of Medicine, University of California Los Angeles

BACKGROUND

Dysregulation of the cell cycle is a hallmark of cancer and can be an important contributor to carcinogenesis (1). LY2835219 is a potent, orally available, highly selective inhibitor of the cyclin dependent kinases (CDK) 4 and 6, blocking phosphorylation of retinoblastoma (Rb) at low nanomolar concentrations. The role of CDK inhibitors for lung cancer, either alone or in combination with other pathway inhibitors, is not clearly defined (2-4). We performed a series of pre-clinical studies to determine the *in vitro* sensitivity to LY2835219 across a panel of 54 molecularly characterized human non-small cell lung cancer (NSCLC) and 10 small cell lung cancer (SCLC) cell lines.

Figure 1. Role of CDK 4/6 in cell proliferation (5-8)



Increasing levels of cyclins and decreasing levels of CDK inhibitors allow for increased assembly of cyclin D-CDK 4/6 complexes. These complexes phosphorylate Rb (pRB), which disassociates from the E2F transcription factor. Once E2F is no longer bound to Rb, E2F can enter the nucleus, where transcription of target genes essential for G1 to S transition occurs. Additionally, tumor suppressor proteins p16 and p21 act as tumor suppressors that are implicated in the prevention of cancers and regulators of cell cycle progression at G1 and S phase.

LY2835219 directly inhibits CDK4/6 kinase activity, preventing Rb phosphorylation.

METHODS

Proliferation Assays: Cells were plated in 24-well plates at a density of 5 x 10^4 to 1 x 10^5 cells per well. The day after plating (day 1), 0-10 µmol/L drug was added. 5 days post treatment, cells were harvested. Viable cells quantified using a Coulter Z2 particle counter (Beckman Coulter Inc.) and the IC_{50} determined based on percent growth inhibition. The 50% inhibitory concentration (IC_{50}) of LY2835219 was determined *in vitro*. Experiments were carried out in duplicate, run at least 2 independent times. Nonlinear curve fitting was conducted by fitting curves to data points using the Proc NLIN function in SAS for Windows version 9.2 (SAS Institute, Inc.) using a basic 4-parameter sigmoid model. Sensitivity to LY2835219 was also evaluated in combination with erlotinib, assessed by serial dilutions of both agents, and serial dilutions of LY2835219 with erlotinib fixed at 1µM in the NSCLC lines.

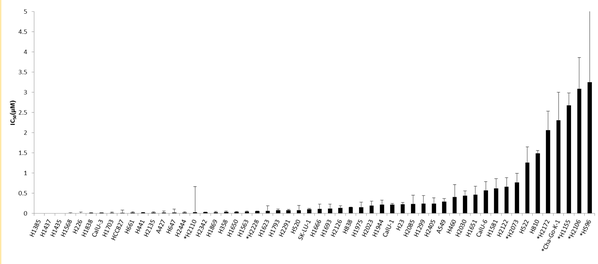
Retinoblastoma (Rb) Status: Rb mutations can be detected by large deletions of the chromosomal region containing the RB1 gene or by deleterious point mutations in RB1. Large deletions were detected by comparative genomic hybridization (CGH), which were performed internally via Agilent arrays. Hybridization of Agilent 105K oligonucleotide CGH arrays were performed according to the manufacturer's protocol for Human Genome CGH 105A Oligo Microarray Kit, Version 5.0 (Agilent Technologies). CGH Analytics software v.4.0 (Agilent Technologies) was used for copy number analysis, employing the ADM2 algorithm (Threshold 5), with Fuzzy Zero and Centralization corrections to minimize background noise. All map positions were based on the March 2006 NCBI36/hg18 genome assembly. A minimum of 3 consecutive probes were required to define a region as amplified or deleted. All data were inspected visually using the interactive view. Exome sequencing (hybrid capture) for point mutations was conducted by the Cancer Cell Line Encyclopedia at the Broad Institute in Cambridge, Massachusetts.

Statistical Analysis: Fisher's exact test was used to determine potential relationships between Rb mutational status and response, as well as between type of lung cancer (small and non-small) and response. The criterion used for significance was a *p*-value of <0.05.

RESULTS

Sensitivity to LY2835219 (defined as an IC_{50} ≤ 500nM) was seen in 43/54 (80%) of the NSCLC cell lines and 3/10 (30%) of the SCLC lines (Figures 2-3, p<0.001). Intact Rb appeared to be required for activity, and inactivated Rb was associated with resistance. 44/54 (81%) of Rb intact lines were sensitive compared to 2/10 (20%) of lines with Rb mutations or loss (Figures 2-3, p<0.001). When examining the response to LY2835219 in specific mutant populations, 79% of the Ras mutant cell lines and 100% of the CDKN2A (the gene that encodes p21) mutant cell lines had an IC_{50} ≤ 500nM (Figure 4). Although synergy was seen with some of the lines treated with erlotinib and LY2835219, in general, it did not appear that the combination, when evaluated in vitro, added much beyond what was seen with the agents individually (Figure 5).

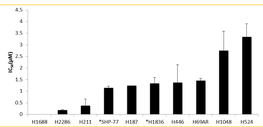
Figure 2: IC_{50} values of NSCLC cell lines with Lilly CDK 4/6 inhibitor (LY2835219).



Sensitivity to treatment is associated with an IC_{50} of ≤ 500nM. Moderate response is an IC_{50} of between 0.5 µM and 1.0 µM. Resistance to treatment is an IC_{50} of >1.0 µM. Error bars, SE based on multiple experiments. * indicates RB point loss or mutation

Figure 3 : IC_{50} values of SCLC cell lines with Lilly CDK4/6 Inhibitor (LY2835219).

* indicates Rb loss or mutation.



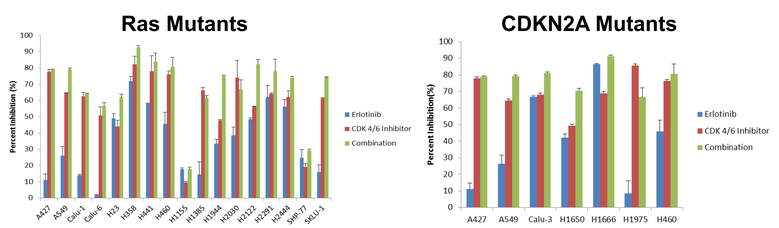


Figure 4. The effects of erlotinib and LY2835219, alone and in combination, on Ras and CDKN2A mutants. Green column, combination; red column, LY2835219 treated; blue column, erlotinib treated. Dosage for erlotinib experiment alone was 1µM and CDK 4/6 inhibitor experiment alone was 100 nM. The combination experiments used 1µM erlotinib and 100 nM LY2835219. Mean of two experiments in duplicate are shown. Error bars, SE based on multiple experiments.

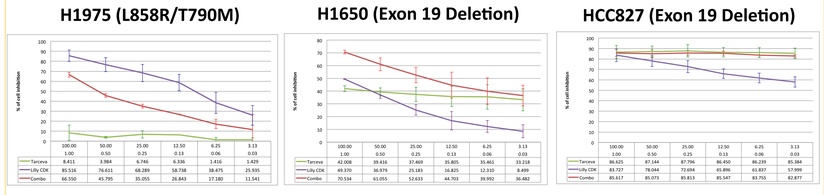


Figure 5: The effects of erlotinib and LY2835219, alone and in combination, on EGFR mutant NSCLC cell lines. Red line, combination; green line, erlotinib treated; purple line, LY2835219 treated. For the erlotinib experiment, the doses were 1.00, 0.50, 0.25, 0.13, 0.06, and 0.03µM. For the LY835219 experiment, the doses were 100, 50, 25,12, 6.25, and 3.13 nM. For the combination experiment, this was conducted by adding 6 dilutions of erlotinib (starting at 1µM) to 6 dilutions of Lilly CDK (starting at 100 nM). HCC827 is the only erlotinib sensitive line.

CONCLUSIONS

. Potent growth inhibition of human lung cancer cell lines with LY2835219 in the majority of cell lines with intact Rb was demonstrated.

. An association between response and Rb status was exhibited.

. CDKN2A as a potential biomarker for predicting response to LY2835219.

. Lack of synergistic effect of erlotinib and LY2835219 indicates that it would be reasonable to test LY2835219 as a single agent, rather than in combination, in NSCLC.

. Comparing these results to the sensitivity of a CDK 4/6 inhibitor in a breast cancer panel is of particular interest, in light of the known clinical activity in that setting (9-10).

ACKNOWLEDGMENTS

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