Selective Antitumor Activity of Ibrutinib in EGFR-Mutant Non–Small Cell Lung Cancer Cells


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Ibrutinib, which irreversibly inhibits Bruton tyrosine kinase, was evaluated for antitumor activity in a panel of non–small cell lung cancer (NSCLC) cell lines and found to selectively inhibit growth of NSCLC cells carrying mutations in the epidermal growth factor receptor (EGFR) gene, including T790M mutant and erlotinib-resistant H1975 cells. Ibrutinib induced dose-dependent inhibition of phosphor-EGFR at both Y1068 and Y1173 sites, suggesting ibrutinib functions as an EGFR inhibitor. Survival was analyzed by Kaplan–Meier estimation and log-rank test. All statistical tests were two-sided. In vivo study showed that ibrutinib statistically significantly suppressed H1975 tumor growth and prolonged survival of the tumor bearing mice (n = 5 per group). The mean survival times for solvent- and erlotinib-treated mice were both 178 days (95% confidence interval [CI] = 14.3 to 213 days), while the mean survival time for ibrutinib-treated mice was 29.8 days (95% CI = 26.0 to 33.6 days, P = .008). Our results indicate that ibrutinib could be a candidate drug for treatment of EGFR-mutant NSCLC, including erlotinib-resistant tumors.


Ibrutinib has been reported to selectively and irreversibly inhibit Bruton tyrosine kinase (BTK) (1,2), which is specifically required for the B-cell antigen receptor signaling pathway (3). Previous studies revealed that ibrutinib specifically inhibited the proliferation of B-cell lymphoma with active B-cell antigen receptor signaling (3) and multiple myeloma cells expressing BTK (4). Oral administration of ibrutinib led to promising in vivo activity against spontaneous B-cell non-Hodgkin lymphoma in dogs and experimental rheumatoid arthritis in mice (1,2). Ibrutinib also inhibited growth of chronic lymphocytic leukemia and multiple myeloma cells inoculated into immune defective mice (4,5). Clinical trials have revealed that ibrutinib is well tolerated and elicits substantial activity in relapsed or refractory B-cell malignancies, with an objective response rate of 60–70% and a complete response of 16–20% (6–8). Ibrutinib was recently approved by the US Food and Drug Administration for treatment of mantle cell lymphoma.

We evaluated antitumor activity of ibrutinib in a panel of non–small cell lung cancer (NSCLC) cell lines and in six- to eight-week-old female nude mice with xenograft tumors derived from H1975 cells. Further details are available in the Supplementary Methods (available online). Animal experiments were carried out in accordance with Guidelines for the Care and Use of Laboratory Animals (NIH publication number 85-23) and the institutional guidelines of the M. D. Anderson Cancer Center. Statistical significance of the differences between treated samples was determined by the two-sided Student t test and one-way analysis of variance (ANOVA). Differences were considered statistically significant at P less than .05. The mean survival time and cumulative survival curve were determined by Kaplan–Meier estimation. The mean survival times were compared by log-rank test. All statistical tests were two-sided.

To test whether ibrutinib can be used for treatment of solid tumors, we evaluated its antitumor activities in a panel of lung cancer cell lines by using a cell viability assay (9) three days after treatment with 0.01 to 30 μM ibrutinib. For the 39 non–small cell lung cancer (NSCLC) cell lines tested, the 50% inhibitory concentration [IC50] of ibrutinib ranged from 0.002 to 30 μM. Among the 39 cell lines tested, 36 had IC50 values between 2 and 30 μM. For the remaining three cell lines, HCC827, H1975, and H292, the IC50 were between 0.002 and 0.195 μM (Figure 1A), all within the clinically achievable concentrations of ibrutinib in the doses used for treatment of lymphoma (6,7), HCC827 and H1975 cells are known to harbor epidermal growth factor receptor (EGFR) mutations, whereas the H292 cell line has wild-type EGFR. Our subsequent analysis showed that EGFR was constitutively active in H292 cells, and that H292 cells were also susceptible to the EGFR inhibitor erlotinib (10). These results suggest that ibrutinib is specific for EGFR-mutant or -constitutively active NSCLC cells.

We next compared erlotinib’s and ibrutinib’s antitumor activities in nine NSCLC cell lines, six of which have mutations or deletions in the EGFR gene. Ibrutinib induced an antitumor spectrum similar to erlotinib in those cell lines, except for the H1975 cells, which harbor a T790M mutation in EGFR and were resistant to erlotinib but susceptible to ibrutinib (Supplementary Table 1, available online). The H1650 cells, which harbor EGFR mutation and PTEN loss (11), were resistant to erlotinib, ibrutinib, and afatinib (IC50 = 2.63 μM), a second generation of EGFR inhibitors that are approved for treatment of EGFR-mutant lung cancer (12). We also compared dose response of ibrutinib and afatinib in H1975 cells. The results showed that ibrutinib induced similar antitumor activity as afatinib in this cell line (Figure 1B).
To test whether ibrutinib can elicit in vivo antitumor activity in EGFR-mutant tumors, we established xenograft tumors from H1975 cells in nude mice and treated mice daily with ibrutinib (25 mg/kg), erlotinib (50 mg/kg), or solvent when tumors reached 4 to 5 mm in diameter. The result showed that treatment with ibrutinib, but not erlotinib, statistically significantly suppressed H1975 tumor growth and prolonged survival of the tumor-bearing mice (Figure 1, C and D). While the mean survival times for solvent- and erlotinib-treated mice were both 17.8 days (95% confidence interval [CI] = 14.3 to 21.3 days), the mean survival time for ibrutinib-treated mice was 29.8 days (95% CI = 26.0 to 33.6 days, \( P = .008 \)), demonstrating in vivo efficacy of ibrutinib in EGFR-mutant cancer.

We determined whether antitumor activity of ibrutinib in NSCLC cells was mediated by inhibition of BTK or by direct effect on EGFR. The expression of BTK was not detectable in any of the cell lines tested (Supplementary Figure 1, available online), indicating that ibrutinib-induced antitumor activity in these cells is not mediated by BTK. In contrast, treatment of H1975 and H3255 cells with erlotinib and ibrutinib led to a similar dose-dependent inhibition of phosphor-EGFR at the Y1173 site in H3255 cells. However, only ibrutinib inhibited pY1068 in H1975 cells (Figure 2A). The basal EGFR phosphorylation at Y1173 was only detectable in HCC827. Like erlotinib, ibrutinib-induced dose-dependent inhibition of EGFR Y1173 phosphorylation in HCC827 cells and constitutive Y1068 phosphorylation in H292 cells, although at relatively higher doses compared with those observed in EGFR-mutant cells. Similar results were observed for EGFR-stimulated Y1068 phosphorylation in A549 cells (Figure 2B), suggesting that at a higher dose, ibrutinib was able to suppress wild-type EGFR activity, consistent with other studies on ibrutinib’s effect on EGFR (1,2).  Ibrutinib-induced inhibition of EGFR phosphorylation occurred as early as 30 min after the treatment (Figure 2C). We also tested whether ibrutinib-induced growth suppression or apoptosis in HCC827 cells. Flow cytometric analysis on apoptotic cells and western blot analyses of poly(ADP-ribose) polymerase (PARP1) and caspase-3 cleavage showed that ibrutinib induced dose-dependent increase of apoptotic cells (42% of apoptotic cells at 72 hours after treatment with 1 \( \mu \)M ibrutinib vs <10% of apoptotic cells in the control group) and cleavage of PARP1 and caspase-3 in HCC827 cells (Figure 2D), demonstrating that apoptosis is the major model of action in HCC827 cells.

EGFR mutations are frequently detected in lung adenocarcinoma patients, especially those who have no smoking history (13,14). The high susceptibilities of EGFR-mutant lung cancer cells to gefitinib and erlotinib (13,15,16) have made these two agents the first choice for treatment of EGFR-mutant
cancers. Unfortunately, despite dramatic responses of EGFR-mutant lung cancer patients to gefitinib or erlotinib, acquired resistance occurs at a median of 10 to 13 months after the treatment initiation (17,18). While a variety of mechanisms have been identified for the acquired resistance, including a second T790M mutation at exon 20 of the EGFR gene (19, 20), amplification of MET gene (21–23), mutations of the KRas gene (24), and activation of AXL or c-Src kinases (25–27), the most common cause of the resistance in clinics is the T790M mutation in the EGFR, which is found in about 50% of those patients (19,21,28). Effort has been made to develop EGFR kinase inhibitors that are effective for EGFR T790M mutants (29–31), including development and approval of afatinib for clinical application (12) and clinical trials on some novel anti-EGFR agents (32). Ibrutinib's selective inhibition of EGFR-mutant NSCLC cells, including the T790M mutant cell line H1975 and its excellent safety profile in patients, indicate that this agent could be a good candidate for treatment of EGFR-mutant NSCLC.

Nevertheless, this study had some limitations, because ibrutinib's inhibitory effect on EGFR was determined on cultured cell lines, not on recombinant EGFR proteins. The differential effects of ibrutinib on wild-type and mutant EGFRs remain to be determined. Moreover, the in vivo study was performed with subcutaneous tumors instead of tumors in orthotopic microenvironments. Because skin rash, a common dose-limiting side effect for EGFR inhibitors (12,33), was observed much less frequently in patients treated with ibrutinib (6–8), it also raises an intriguing question on whether ibrutinib can be used as an EGFR inhibitor to treat cancers in clinics.

Figure 2. Western blot analysis of epidermal growth factor receptor (EGFR) phosphorylation (p-EGFR) and cleavage of poly(ADP-ribose) polymerase (PARP) and caspase 3 (Casp-3). A) H1975 and H3255 cells were treated with erlotinib and ibrutinib at the doses as indicated. Phospho-Y1068 and total EGFR were determined in 24 hours after treatment. B) HCC827, H292, and A549 cells were treated with erlotinib or ibrutinib at the doses as indicated. Cell lysates were harvested for protein phosphorylation analyses at 24 hours. Note, for A549 cells, 10 ng/ml EGF was added to the cells to activate EGFR 15 min before harvesting cells. C) HCC827 cells were treated with 0.5 μM erlotinib or 0.5 μM ibrutinib and tested for EGFR phosphorylation at different time points as indicated. D) HCC827 cells were treated with ibrutinib and tested for EGFR phosphorylation and caspase-3 and PARP1 cleavages at 48 hours.

References


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