Barasertib (AZD1152), a Small Molecule Aurora B Inhibitor, Inhibits the Growth of SCLC Cell Lines In Vitro and In Vivo

Barbara A. Helfrich, Jihye Kim, Dexiang Gao, Daniel C. Chan, Zhiyong Zhang, Aik-Choon Tan, and Paul A. Bunn Jr

Abstract

Small-cell lung cancer (SCLC) cells have rapid proliferation, universal Rb inactivation, and high rates of MYC family amplification, making aurora kinase inhibition a natural target. Preclinical studies have demonstrated activity for Aurora A and pan-Aurora inhibitors with some relationship to MYC family expression. A clinical trial showed activity for an Aurora kinase A inhibitor, but no biomarkers were evaluated. We screened a panel of 23 SCLC lines with and without MYC family gene amplification or high MYC family gene expression for growth inhibition by the highly potent, selective aurora kinase B inhibitor barasertib. Nine of the SCLC lines were very sensitive to growth inhibition by barasertib, with IC_{50} values of <50 nmol/L and >75% growth inhibition at 100 nmol/L. Growth inhibition correlated with cMYC amplification (P = 0.018) and cMYC gene expression (P = 0.026). Sensitive cell lines were also enriched in a published MYC gene signature (P = 0.042). In vivo, barasertib inhibited the growth of xenografts established from an SCLC line that had high cMYC gene expression, no cMYC amplification, and was positive for the core MYC gene signature. Our studies suggest that SCLC tumors with cMYC amplification/high gene expression will frequently respond to Aurora B inhibitors and that clinical studies coupled with predictive biomarkers are indicated. Mol Cancer Ther; 15(10): 2314–22. ©2016 AACR.

Introduction

Small-cell lung cancer, accounting for approximately 35,000 cases annually, is the sixth most common cause of cancer deaths in the United States (1). Clinically, SCLC tumors have rapid doubling times and a propensity for early development of widespread metastatic disease (2). The overall 5-year survival from diagnosis is less than 5% despite high initial response rates to first-line chemotherapy of 70% to 90% (2, 3).

There have been few therapeutic advances in the treatment of SCLC in recent decades, and SCLC remains a major public health problem. The standard of care for both limited stage (IIB–III) and extensive stage (IV) has been the drug combination of etoposide–cisplatin or carboplatin since the early 1980s (4). Modest improvements in survival have come from the addition of chest radiotherapy in limited stage. Studies evaluating new-generation cytotoxic chemotherapies have not had a significant impact on the standard of care, and topotecan is the only cytotoxic currently approved for the treatment of relapsed SCLC (5, 6). Biological studies have shown that loss of the RBL1 and TP53 tumor suppressor genes is universally present in SCLC cells (7, 8). In addition, a significant proportion of SCLCs have amplification of various MYC family members (9).

A recent biological approach to cancer has been the development of small molecules targeting the key mitotic regulatory serine/threonine kinases Aurora A (AURKA) and Aurora B (AURKB), which are frequently overexpressed in lung cancer (10, 11). During mitosis, AURKA and AURKB coordinate cell-cycle progression through G2-M. AURKA regulates centrosome maturation and separation, bipolar spindle assembly, and mitotic entry (12). AURKB plays a critical role by regulating chromosome alignment, accurate segregation, and cytokinesis by its movement through the mitotic stages (12). In a human colon carcinoma cell line, AURKB inhibition by barasertib resulted in Rb hypophosphorylation, leading to polyploidy after an aberrant mitosis (13). The phenotypic result of AURKB inhibition is an induction of polyploidy, a hallmark of anti-tumor activity.

Currently, aurora kinase inhibitors are in clinical trials; however, predictive biomarkers for patient selection are needed (10). In a recent pharmacologic screen of 34 SCLC lines for growth inhibition by the AURKA inhibitor MLN8237 and the dual Aurora A/B inhibitors PHA680632, VX680, and ZM44739, six SCLC lines that had 50% growth-inhibitory concentrations (IC_{50}) of <1 nmol/L to all four drugs were considered sensitive, and response was correlated with amplification of the cMYC oncogene (14). However, there were several lines with cMYC amplification that did not respond and several other lines without cMYC amplification that were sensitive. Furthermore, amplification of...
MYC family members MYCL1 and MYCN did not correlate with sensitivity to dual Aurora A/B inhibitors or the AURKA inhibitor MLN8237 (14). A phase 1 clinical trial reported activity of MLN8237 in 21% of relapsed SCLC patients; however, cMYC expression was not evaluated (15).

In contrast, growth inhibition by the dual Aurora A/B inhibitor PF-03814735 in a panel of 20 SCLC lines correlated with amplification or overexpression of any of the MYC family members (cMYC, MYCL1, and MYCN; ref. 16). In this study, 14 SCLC lines with IC50s <100 nmol/L were considered resistant. Again, there were SCLC lines with no evidence of MYC family amplification or overexpression that were sensitive to PF-03814735. Resistance was defined as an IC50 of >3 µmol/L, and no MYC family amplification was found in these resistant lines. PF-03814755 inhibited the in vitro growth of cMYC and MYCN-amplified cell lines in SCLC tumor xenograft models (16). These studies suggest that there is some link between MYC family members and the Aurora kinases A and B in SCLC, but no studies of specific aurora kinase B inhibitors have been reported.

MYC family gene amplification in conjunction with mutation/deletion of the tumor suppressor genes TP53 and RB1 are the most frequently altered genes in SCLC (7–9). Focal amplification of the MYC family of transcription factors, including cMYC, MYCL1, and MYCN, has been found in about 30% of SCLC samples, and amplification of MYCL1 and MYCN are found exclusively in neuroendocrine tumors, including SCLC (17). A recent report using chromogenic in situ hybridization evaluated cMYC amplification in 77 formalin-fixed paraffin-embedded tumor samples from SCLC patients who had a diagnostic biopsy for SCLC (18). cMYC amplification was found in 20% of the biopsies and associated with poor survival. Furthermore, p53 proteins with missense mutations have been shown to transactivate cMYC through the C-terminus (19).

In both of the above reports, the associated phenotypic drug induced changes by the aurora kinase inhibitors, including increased G2–M arrest, polyploidy, and a decrease in histone H3 phosphorylation, were thought to be primarily due to inhibition of AURKB (14, 16). We therefore evaluated barasertib, an AURKB-specific inhibitor, in a panel of 23 SCLC lines with and without MYC family amplification.

Materials and Methods

Reagents

Barasertib and barasertib-HQPA were provided by AstraZeneca Pharmaceuticals. The structure of barasertib was previously published (20). Barasertib is rapidly converted by plasma phosphatases to the active barasertib-HQPA metabolite. Therefore, in vitro studies were conducted with barasertib-HQPA and in vivo experiments with barasertib (20). The barasertib-HQPA IC50 for AURKB is 0.37 nmol/L versus 1.369 nmol/L for AURKA (20).

SCLC cell lines

The SCLC lines H82, H114, H187, H211, H345, H378, H446, H524, H526, H748, H774, H841, H889, H1092, H11694, H11963, H2029, H2081, H2141, H2171, and N417 were obtained from Drs. Adi Gazdar and John Minna (UT Southwestern, Dallas, TX) and deposited in the UCCCC Tissue Culture Core. Authentication of the cell lines by short tandem repeat DNA profiling was through the Tissue Culture Core and the UCCCC DNA Sequencing and Analysis Core. The DMS114 and H69 lines were obtained from the ATCC. All cell lines were used within 6 months of resuscitation of a frozen vial from the authenticated passage. The clinical characteristics of the patients from whom the cell lines were derived are shown in Table 1 (21). All cell lines were mycoplasma free and maintained in RPMI1640 + 5% or 10% FBS at 37°C in 5% CO2. The cell lines were obtained in 1993 (H146, H187, H211, H345, H378, H446, H524, H526, H748, H774, and H841), 2011 (DMS114), and 2014 (H69, H82, H889, H1092, H11694, H11963, H2029, H2081, H2141, H2171, and N417).

MYC family gene amplification, gene expression, and MYC core gene signature

Cell line MYC family amplification (14, 16), MYC family gene expression (Cancer Cell Line Encyclopedia, accession number GSG36133; broadinstitute.org), and the MYC core gene signature (Gene Expression Omnibus, accession number GSE15523; ncbi.nlm.nih.gov; ref. 22) were used to determine whether they related to cell line sensitivity to barasertib.

Aurora A/B gene and Aurora B protein expression by Western blotting

AURKB and AURRKB gene expression in our panel of 23 SCLC lines is shown in Supplementary Fig. S1A and S1B. Gene expression data was from the CCLE (accession number GSE36133) and was significantly higher than in 17 normal epithelial airway samples (accession number GSE24337; P < 0.001). We confirmed AURKB protein expression by Western blotting in a subset of SCLC lines (Supplementary Fig. S1C). Cell lysates were prepared in Triton X-100 lysis buffer, sonicated, and protein concentration determined by DC Protein Assay (Bio-Rad). Protein (50 µg) was resolved by SDS-PAGE. Gels were electroblotted onto PVDF membranes and probed for AURKB (Cell Signaling Technology). Protein bands were visualized by chemiluminescence using SuperSignal West Femto (Thermo Scientific). β-Actin (Cell Signaling Technology) served as a loading control. AURKB protein expression was similar across the SCLC lines.

Modified tetrazolium growth assays

Inhibition of cell growth was assessed using MTS (CellTiter Aquous One Solution, Promega; ref. 23). Briefly, 2 x 103 to 1 x 104 viable cells were plated in growth medium in 96-well plates (Corning) and incubated overnight at 37°C. Barasertib-HQPA, 0 to 100 nmol/L, was added, and plates were incubated for 5 days, after which the MTS was added and the absorbency of each well was measured at 490 nm using an automated plate reader (Molecular Devices).

Immunofluorescence staining phosphorylated histone H3

Phosphorylated histone H3 (Ser10) expression was determined by FACS analysis. Briefly, cells were fixed with 4% formaldehyde at 37°C for 10 minutes and then chilled on ice for 1 minute. Fixed cells were permeabilized on ice for 30 minutes in ice-cold 90% ethanol and then blocked in 0.5% BSA for 10 minutes at room temperature. The fixed permeabilized block cells were stained for 60 minutes at room temperature, followed by three washes. Primary antibody was a rabbit mAb to phosphorylated histone H3 (Ser10) directly conjugated with Alexa Fluor 488 (Cell Signaling Technology), and the isotype control was a rabbit IgG directly conjugated with Alexa Fluor 488 (Cell Signaling Technology). Cell fluorescence was measured by FACS.

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Table 1. Cell line characteristics

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Abbreviations: E, extensive stage; F, female; GE, gene expression; L, limited stage; M, male; PY, pack year; Unk, unknown.

Footnotes:

*Data obtained from ref. 20.

**MYC family amplification status obtained from refs. 15 and 17.

***MYC family gene expression was obtained from the Cancer Cell Line Encyclopedia (broadinstitute.org).

(From Beckman FC500, Beckman Coulter). The percentage of positive phosphorylated histone H3 (Ser10) cells was determined using the Coulter software.

**Ploidy analysis**

Briefly, 3 x 10^5 cells were exposed to barasertib-HQPA 30 or 50 nmol/L for 24 and 48 hours and then stained with saponin, propidium iodide (PI), and RNase solution overnight. Alterations in DNA ploidy were determined by flow cytometry. Doublet cells were gated out of the analysis. Remaining cells were analyzed on a log2 scale to determine the percentage of the population that was tetraploid (DNA content 4N) and the subsequent accumulation of polyploid cells (DNA content >8N).

**Nude mouse xenograft tumor model**

Athymic nude mice (4- to 6-week-old females) obtained from the NCI (Bethesda, MD) were maintained in the University of Colorado Denver Animal Resources Center (Denver, CO) with an approved protocol by IACUC. The SCLC line H841 was mixed with an equal volume of cold Matrigel and implanted at 5 x 10^6 cells in 100 μL in the rear flank. Treatment began on day 20 after tumor cell implantation. Mice were randomized into 8 mice per group, and 5 to 7 of these that developed tumors were treated with vehicle, barasertib at 50 or 100 mg/kg per day for 5 days, rested 5 to 7 of these that developed tumors were treated with barasertib-HQPA. The growth inhibition IC50 concentration for all 9 lines was <50 nmol/L, and at 100 nmol/L, barasertib-HQPA growth inhibition was >75%. cMYC was amplified in 5 of these lines, 2 lines were MYCL1 gene amplified, and 2 lines had no MYC family amplification. The 10 SCLC lines shown in Fig. 1 were classified as having intermediate sensitivity to barasertib-HQPA, as growth inhibition at 100 nmol/L was 32% to 50%. Increasing the barasertib-HQPA concentration to 1 μmol/L did not increase growth inhibition (data not shown). One cell line with intermediate sensitivity was cMYC amplified. 4 lines were MYCL1 amplified, 2 lines had MYCN gene amplification, and 3 lines had no MYC family amplification.

As shown in Fig. 1, we found three patterns of growth inhibition. Pattern A (Fig. 1A) included 9 SCLC lines that were the most sensitive to barasertib-HQPA. The growth inhibition IC50 concentration for all 9 lines was <50 nmol/L, and at 100 nmol/L, barasertib-HQPA growth inhibition was >75%. cMYC was amplified in 5 of these lines, 2 lines were MYCL1 gene amplified, and 2 lines had no MYC family amplification.

**Results**

**In vitro growth inhibition by barasertib-HQPA**

We evaluated the growth-inhibitory effects of barasertib-HQPA on cMYC-amplified, 7 MYCL1 amplified, 2 MYCN amplified, and 8 SCLC lines with no MYC family amplification (Table 1). Barasertib-HQPA concentrations evaluated were 0 to 100 nmol/L, ensuring that AURKA was not inhibited. The barasertib-HQPA concentrations were similar to those used in other preclinical studies and exceeded the trough levels achieved in human trials (24, 25).

As shown in Fig. 1, we found three patterns of growth inhibition. Pattern A (Fig. 1A) included 9 SCLC lines that were the most sensitive to barasertib-HQPA. The growth inhibition IC50 concentration for all 9 lines was <50 nmol/L, and at 100 nmol/L, barasertib-HQPA growth inhibition was >75%. cMYC was amplified in 5 of these lines, 2 lines were MYCL1 gene amplified, and 2 lines had no MYC family amplification.

The 4 SCLC lines shown in Fig. 1B were classified as having intermediate sensitivity to barasertib-HQPA, as growth inhibition at 100 nmol/L was 32% to 50%. Increasing the barasertib-HQPA concentration to 1 μmol/L did not increase growth inhibition (data not shown). One cell line with intermediate sensitivity was cMYC amplified. 4 lines were MYCL1 amplified, 2 lines had MYCN gene amplification, and 3 lines had no MYC family amplification.

The 4 SCLC lines shown in Fig. 1C were classified as resistant to barasertib-HQPA, as growth inhibition at 100 nmol/L was <20%, and increasing the concentration to 1 μmol/L did not
alter the growth inhibition (data not shown). Three resistant lines had no MYC family amplification and 1 line was MYCL1 gene amplified.

Relationship of barasertib-HQPA growth inhibition to MYC family gene amplification

As shown in Fig. 1A–C, 6 cell lines were cMYC amplified, of which 5 were sensitive. Seventeen cell lines were not cMYC amplified, of which 4 were sensitive. Thus, there was a significant association between sensitivity to growth inhibition by barasertib-HQPA and cMYC amplification (P = 0.018). The odds of being sensitive were 16 times higher [95% confidence interval (CI), 1.4, 183] for cMYC-amplified SCLC lines compared with non-cMYC–amplified cell lines. The association between MYCL1 gene amplification and growth inhibition was not significant (P = 0.2). As there were only 2 MYCN-amplified lines and both had intermediate sensitivity, we did not evaluate this as a separate group. There was also no significant association between sensitivity to barasertib-HQPA and having any MYC family gene amplification (P = 0.4).

Relationship of barasertib-HQPA growth inhibition to cMYC gene expression

We next determined whether there was an association between cMYC gene expression and sensitivity to growth inhibition by barasertib-HQPA (Table 1; Fig. 2A). The mean cMYC gene expression in sensitive lines (10.9 ± 4) was higher than the mean cMYC gene expression of the intermediate/resistant lines (7.2 ± 3.3). The cMYC gene expression was high (>10) in 11 cell lines, of which 7 were sensitive, and the cMYC gene expression was low (<7.5) in 12 cell lines, of which 2 were sensitive to barasertib-HQPA. This difference was significant (P = 0.026).

There was no significant association between the mean MYCL1 gene expression in the sensitive cell lines (8.4 ± 2.7) and the intermediate/resistant lines (9.8 ± 2.8; P = 0.28). There was also no significant association between the mean MYCN gene expression in the sensitive cell lines (5.3 ± 0.67) and the intermediate/resistant lines (6.7 ± 3; P = 0.13).

We then separated the cell lines into two groups based on cMYC gene expression modeled as a categorical variable based on quartile cut-off values of the initial distribution of being sensitive, intermediate, or resistant to growth inhibition by barasertib-HQPA. Categories were combined if similar coefficients were observed. The best functional form of cMYC gene expression was the dichotomized variable, with two categories at the cut-off point of 12.9 (<12.9 vs. ≥12.9), which corresponded to the 75th percentile of the cMYC gene. On the basis of these criteria, the odds of being sensitive were 11 times higher (95% CI, 1.2, 103) for cell lines with cMYC gene expression ≥12.9 compared with cell lines with cMYC gene expression <12.9.

As shown in Fig. 2B, we analyzed our cell line panel for the expression of a core MYC gene signature (22). The cMYC signature was present in 9 cell lines, of which 6 were sensitive to barasertib-HQPA. The cMYC gene signature was absent in 14 cell lines, of
which 3 were sensitive to barasertib-HQPA. All 5 cMYC gene amplified lines that were sensitive to growth inhibition by barasertib-HQPA expressed the core MYC gene signature, as did the H524 cMYC-amplified line, which was intermediate in growth inhibition. None of the 7 MYCL1-amplified lines expressed the core MYC gene signature, including the H378 and H1963 lines, which were sensitive to growth inhibition. The 2 MYCN-amplified lines were both intermediate to growth inhibition, and H526 was positive for the core signature and H69 was negative. Two of the 8 SCLC lines that did not have MYC family gene amplification were positive for the MYC core gene signature, and the H841 line was sensitive to growth inhibition and DMS114 was intermediate. The remaining 6 SCLC lines without MYC family amplification were negative for the core MYC gene signature. The H2081 line was sensitive to growth inhibition by barasertib-HQPA, and the remaining 5 lines were intermediate or resistant to growth inhibition. There was a statistically significant correlation between expression of the MYC core gene signature and sensitivity to growth inhibition by barasertib-HQPA ($P = 0.042$), although this correlation was not superior to the use of cMYC gene expression ($P = 0.026$) or amplification ($P = 0.018$).

**Barasertib-HQPA inhibited phosphorylation of histone H3**

To ensure that resistance to growth inhibition by barasertib-HQPA was not due to lack of drug uptake by the cell lines, we evaluated phosphorylated histone H3 (Ser10) by flow cytometry. Phosphorylation on histone H3 (Ser10) by AURKB is required for chromosome condensation during mitosis (26). The fraction of detectable cells with phosphorylated H3 (Ser10) was very low in untreated SCLC lines (data not shown). Therefore, we treated the SCLC lines with paclitaxel for 24 hours to arrest the cells in the $G_2-M$ phase of the cell cycle, increasing the fraction of cells with phospho-H3 (Ser10). As shown in Fig. 3, a concurrent 24-hour treatment with paclitaxel and barasertib-HQPA reduced the fraction of phosphorylated H3 (Ser10)-positive cells induced by paclitaxel alone. This demonstrated that barasertib-HQPA actively inhibited the kinase activity of AURKB in cell lines both sensitive (Fig. 3A, H446) and resistant (Fig. 3B and C, H345 and H748) to barasertib-HQPA–induced growth inhibition, suggesting that drug uptake does not explain the difference. Although it is possible that barasertib-HQPA could be less stable in the resistant lines during a longer exposure, it has been shown to be stable for >72 days at pH 7.4 and the extrapolated half-life at pH 1 is >11.4 days (personal communication on July 12, 2016, Dr. Sue Ashton, AstraZeneca).

**Barasertib-HQPA induced polyploidy**

The induction of polyploidy (DNA content ≥8N) is the hallmark of phenotypic changes induced by AURKB inhibition as the cells reenter the S-phase of the cell cycle without going through cytokinesis (27). We evaluated the degree of polyploidy at 24 and 48 hours posttreatment with 30 or 50 nmol/L barasertib-HQPA in 10 SCLC lines, representing lines with cMYC, MYCL1, MYCN...
amplification, and no MYC family amplification, that were sensitive, intermediate, or resistant to growth inhibition by barasertib-HQPA (Table 2).

After exposure to 30 nmol/L barasertib-HQPA, the sensitive cell lines H378, H841, H211, and H446 had a significant increase in the fraction of 4N cells at 24 hours and the fraction of 8N cells at 48 hours (Table 2). Similarly, in the intermediate lines H69, H146, H187, and H524 the fraction of 4N cells was significantly increased at 24 hours, and the fraction of 8N cells increased at 48 hours.

While the time course for the appearance of a tetraploid peak followed by a polyploid peak was consistent across the above cell lines, this was not the scenario in the more resistant lines. The H345 cell line was initially treated with 30 nmol/L barasertib-HQPA, and as shown in Table 2, despite an increase in the fraction of 4N cells, there was no increase in the 8N cell fraction. Subsequently, we treated H345 and the other resistant cell lines DMS53, H774, and H748 with 50 nmol/L barasertib-HQPA; as shown in Table 2, the cell lines remained 4N following 48 hours of treatment. In summary, cytokinesis failure in the cell lines sensitive and intermediate to growth inhibition by barasertib-HQPA resulted in DNA endoreduplication and the appearance of polyploid cells (DNA content 8N). In contrast, the resistant cell lines H345, DMS53, H774, and H748 remained in a tetraploid state, did not reenter the S-phase, and did not undergo endoreduplication.

Barasertib inhibited tumor xenografts

We evaluated the in vivo efficacy of barasertib on the SCLC line H841 (no cMYC family amplification, high cMYC gene expression, and positive for the MYC gene expression signature). In the in vitro growth inhibition assays described above, we demonstrated that 100 nmol/L barasertib-HQPA inhibited the growth of H841 by 85%. The H841 cells were implanted subcutaneously in nude mice and treated with barasertib at 50 or 100 mg/kg/day Monday to Friday for 2 weeks. The barasertib doses were within the range previously published for in vivo studies of solid tumors and provided trough barasertib-HQPA serum concentrations above the concentrations used in vitro (28, 29).

As shown in Fig. 4A, the H841 xenografts were significantly growth inhibited during the 2-week barasertib treatment (days 20–31) compared with control mice. On day 34, the control mice were sacrificed due to tumor size. On day 34, the control tumors had a mean tumor volume of 2,774 ± 2,106 mm³ compared with 232 ± 186 mm³ in the 50 mg/kg treatment group (P = 0.011). The treatment groups were followed for an additional 30 days following cessation of treatment. At day 61, the mean tumor volume in the 50 mg/kg treatment group was 2,828 ± 3,670 mm³, comparable with the growth of the control tumors at sacrifice.

The 100 mg/kg barasertib dose caused H841 tumor xenograft regression, and the tumors remained regressed through day 61 when the experiment was terminated. There was no loss of weight throughout the course of treatment in treated animals compared with control mice (Fig. 4B).

Discussion

In this study, we show that the specific AURKB inhibitor barasertib-HQPA has growth-inhibitory effects in some SCLC lines and that cMYC amplification or high gene expression or MYC gene signature is a useful predictive biomarker. There has been little advance in the therapy of SCLC over the past three decades, and new therapeutic options are urgently needed. The rapid growth and frequent overexpression of aurora kinases, which are key regulators of mitosis, has made aurora kinase inhibitors attractive targets for SCLC therapy. AURK directly phosphorylates Rb at serine 780, playing a critical role in regulating postmitotic checkpoints to prevent polyploidy after an aberrant mitosis (13). In SCLC, loss of Rb and p53 function is essentially universal, leading to suppression of postmitotic checkpoints that prevent polyploidy after aberrant mitosis by eliciting a pseudo G1 arrest. Barasertib-HQPA was previously reported to induce polyploidy in tumor cells, providing rationale for the study of barasertib-HQPA in SCLC (13). We report that polyploidy is induced in sensitive line but not in resistant lines.

Prior studies with dual aurora kinase A/B inhibitors and with specific AURKA kinase inhibitors demonstrated considerable activity in a small number of SCLC cell lines (14, 16). Growth
inhibition by dual aurora A/B inhibitors and with AURKB knockdown has been correlated with cMYC amplification alone (14) and by other investigators with any MYC family amplification or high MYC family gene expression (16). Recently, it has also been shown through activity-based protein profiling that AURKB is a critical kinase in cMYC-amplified SCLC cell lines but not in SCLC lines that lack cMYC amplification (30). Amplifications and overexpression of MYC family oncogenes has been reported in 15% to 30% of SCLCs (17, 18).

We report that 39% of the cell lines tested were sensitive to growth inhibition by barasertib-HQPA at concentrations that can be achieved in humans and do not inhibit AURKA. We found that cMYC amplification, cMYC gene expression, and a cMYC gene signature were significant predictors of growth inhibition to barasertib-HQPA. The strongest association between growth inhibition was cMYC gene amplification (P = 0.018), but cMYC gene expression (P = 0.026) and cMYC gene signature (P = 0.042) also correlated with growth inhibition. In addition, by an OR estimate, cMYC-amplified cell lines were 16 times more likely to be sensitive to growth inhibition by barasertib-HQPA than non-cMYC-amplified cell lines. For cMYC gene expression, the odds of being sensitive to growth inhibition were 11 times higher for cell lines with cMYC gene expression >12.9. Although the correlations between cMYC amplification, cMYC gene expression and cMYC gene signature, and response to barasertib-HQPA are significant, the sample size is small and should be confirmed in clinical trials where cMYC amplification status is assessed in all patients. Although there was some relationship to expression/amplification of other MYC family members and sensitivity to barasertib-HQPA, these correlations were not significant. However, in both our study and the studies of Sos and colleagues, there were cMYC-amplified cell lines that were not sensitive to growth inhibition by AURKB or dual aurora kinase A/B inhibitors (14).

In contrast, Hook and colleagues found significant association between IC50 values for the dual AURKA/AURKB inhibitor PF-3814735 and activation/amplification of any MYC family member (P = 0.001; ref. 16). We did not observe this with MYCL1 amplification (P = 0.2). There was also no significant association between sensitivity and being amplified for any MYC family member (P = 0.4). We also did not find a statistically significant association between high MYCL1 (P = 0.28) or MYCN (P = 0.12) gene expression and sensitivity to growth inhibition.

Further support of the potential combined predictive power of cMYC amplification/high gene expression and the MYC core gene signature is evidenced by our in vivo experiments. We implanted the SCLC line H841, which has high cMYC gene expression, no MYC family amplification, but was positive for the core MYC gene signature. This line was sensitive in vitro to growth inhibition by barasertib-HQPA, IC50 < 50 nmol/L. Mice were dosed with either 50 or 100 mg/kg of the barasertib. The 50 mg/kg dose inhibited tumor growth; however, when treatment was stopped, the tumors began growing. At the 100 mg/kg dose, the H841 tumor regressed and remained regressed until the termination of the experiment.

Previous investigators evaluated the dual aurora A/B inhibitor PF-03814735 in mice-bearing xenografts of the cMYC-amplified SCLC line H82 and the MYCN-amplified SCLC line H69 (16). Growth inhibition in the cMYC-amplified H82 xenograft model was greater than in the MYCN H69-amplified xenografts. In our in vivo investigation of the efficacy of barasertib-HQPA, the cMYC-amplified line H82 was sensitive to growth inhibition (IC50 < 50 nmol/L), and the H69 MYCN-amplified line had only intermediate sensitivity, as at 100 nmol/L barasertib-HQPA, growth inhibition was 43%. The cMYC-amplified line H82 was positive for the core MYC gene signature and the MYCN-amplified H69 line was not.

There are some clinical data suggesting a role for aurora kinase inhibitors in SCLC. A phase II study of alisertib (AURKA inhibitor) given twice daily for 7 days every 21 days showed a response rate of 21% among 48 previously treated SCLC patients (15). Unfortunately, no biomarker correlates, such as cMYC amplification or gene expression, were determined. This alisertib phase II study was followed by a randomized global phase II trial of alisertib plus weekly paclitaxel versus placebo plus weekly paclitaxel in the second-line setting. This trial has completed accrual, but the results have not been reported. Unfortunately, cMYC amplification was not evaluated in this study. Clinical studies of alisertib

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**Table 2.** Percent ploidy induced by barasertib-HQPA.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Conc. nmol/L</th>
<th>Time</th>
<th>4N ≥8N</th>
<th>%Gl 30 nmol/L at 120 h</th>
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<tbody>
<tr>
<td>H446</td>
<td>30</td>
<td>0 h</td>
<td>31</td>
<td>6</td>
</tr>
<tr>
<td>cMYC</td>
<td></td>
<td>24 h</td>
<td>75</td>
<td>10</td>
</tr>
<tr>
<td>Amp</td>
<td></td>
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<td>24 h</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>cMYC</td>
<td></td>
<td>30</td>
<td>80</td>
<td>5</td>
</tr>
<tr>
<td>Amp</td>
<td></td>
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<tr>
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<td>25</td>
<td>0</td>
</tr>
<tr>
<td>No Amp</td>
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<td>24 h</td>
<td>61</td>
<td>16</td>
</tr>
<tr>
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<td>0 h</td>
<td>24 h</td>
<td>80</td>
<td>48</td>
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<td>24 h</td>
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<td>0</td>
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<tr>
<td>Amp</td>
<td></td>
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<td></td>
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<tr>
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</tr>
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<td>24 h</td>
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<td>1</td>
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<td>24 h</td>
<td>44</td>
<td>3</td>
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<tr>
<td>Amp</td>
<td></td>
<td>48 h</td>
<td>55</td>
<td>8</td>
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Abbreviations: 4N, tetraploid DNA content; >8N, ploidy DNA content; h, hour; Amp, amplification; Conc., concentration; Gl, growth inhibition; ND, not done.
and other aurora kinase inhibitors have generally used a continuous dosing schedule largely based on preclinical studies with leukemia models. The early clinical studies using continuous dosing schedules reported myelosuppression as the dose-limiting toxicities (15, 24, 25). In our studies, SCLC cell lines were growth inhibited with a single exposure in vitro and an intermittent exposure in vivo.

Recently, AstraZeneca developed nanoparticles containing AZD2811 formerly known as barasertib-HQPA that increases biodistribution to tumor sites with minimal impact on bone marrow pathology, resulting in lower toxicity and increased efficacy in multiple tumor models at half the dose intensity of barasertib (31). Thus, we believe that SCLC clinical trials with aurora kinase inhibitors, such as AZD2811, using a panel of MYC biomarkers and intermittent drug scheduling studies should be done where a high dose is given intermittently, as this is maybe more effective and would allow recovery of the bone marrow. Support of an intermittent dosing schedule was also supported by an in vivo xenograft study using the cMYC-amplified H82 SCLC line, where tumor growth control was more effective with a once weekly high dose 80 mg/kg (2 × daily once for 3 weeks) of the dual A/B PF-03814735 inhibitor compared with a low-dose daily schedule using 15 mg/kg (2 × daily for 10 conservative days; ref. 16). Furthermore, a high-dose short course of barasertib therapy in a xenograft flank model using a MYCN-overexpressing medulloblastoma model was efficacious at reducing tumor volume. Mice were dosed with barasertib at 50 mg/kg 2 × daily for 2 days, and tumor volumes were significantly different from 4 days after treatment until the end of the experiment (P < 0.05; ref. 32). We believe that an AZD2811 clinical trial should be considered in SCLC patients who progress after etoposide/platinum doublet therapy with assessment of cMYC amplification and gene expression in all patients to determine the response rate in SCLC patients with and without cMYC amplification. Alternatively, a "window of opportunity" maintenance trial could be done after 4 cycles of etoposide/cisplatin to determine time to progression in patients with and without cMYC amplification.

In conclusion, the AURKB inhibitor, barasertib-HQPA, inhibited the growth of SCLC lines both in vitro and in vivo. Growth inhibition was greater in cell lines where barasertib-HQPA induced endoreduplication. Our studies suggest that SCLC tumors with cMYC amplification/high gene expression will frequently respond to Aurora B inhibitors and that clinical studies of the novel AZD2811 nanoparticle coupled with predictive biomarkers are indicated especially using an intermittent dosing schedule.

Disclosure of Potential Conflicts of Interest

P.A. Bunn reports receiving a commercial research grant from and is a consultant/advisory board member for AstraZeneca. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: B.A. Helfrich, D.C. Chan, P.A. Bunn Jr
Development of methodology: B.A. Helfrich
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): B.A. Helfrich, D.C. Chan
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): B.A. Helfrich, J. Kim, D. Gao, D.C. Chan, A.-C. Tan
Writing, review, and/or revision of the manuscript: B.A. Helfrich, J. Kim, D. Gao, A.-C. Tan, P.A. Bunn Jr
Study supervision: P.A. Bunn Jr
Other (animal work): Z. Zhang

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References
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Barbara A. Helfrich, Jihye Kim, Dexiang Gao, et al.


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