Characterization of the Novel and Specific PI3Kα Inhibitor NVP-BYL719 and Development of the Patient Stratification Strategy for Clinical Trials

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Abstract

Somatic PIK3CA mutations are frequently found in solid tumors, raising the hypothesis that selective inhibition of PI3Kα may have robust efficacy in PIK3CA-mutant cancers while sparing patients the side-effects associated with broader inhibition of the class I phosphoinositide 3-kinase (PI3K) family. Here, we report the biologic properties of the 2-aminothiazole derivative NVP-BYL719, a selective inhibitor of PI3Kα and its most common oncogenic mutant forms. The compound selectivity combined with excellent drug-like properties translates to dose- and time-dependent inhibition of PI3Kα signaling in vivo, resulting in robust therapeutic efficacy and tolerability in PIK3CA-dependent tumors. Novel targeted therapeutics such as NVP-BYL719, designed to modulate aberrant functions elicited by cancer-speciﬁc genetic alterations upon which the disease depends, require well-deﬁned patient stratification strategies in order to maximize their therapeutic impact and beneﬁt for the patients. Here, we also describe the application of the Cancer Cell Line Encyclopedia as a preclinical platform to reﬁne the patient stratification strategy for NVP-BYL719 and found that PIK3CA mutation was the foremost positive predictor of sensitivity while revealing additional positive and negative associations such as PIK3CA ampliﬁcation and PTEN mutation, respectively. These patient selection determinants are being assayed in the ongoing NVP-BYL719 clinical trials. Mol Cancer Ther; 13(5); 1117–29. ©2014 AACR.

Introduction

Phosphoinositide-3 kinases (PI3K) are widely expressed lipid kinases that function as key signal transdu-
PIK3CA one of the most commonly mutated oncogenes in human cancers. Although PIK3CA mutations can be detected across the entire coding sequence of the gene, 80% of the mutations are found in 3 major hotspot clusters in the helical (E542K, E545K) and kinase domains (H1047R). Each of these mutations leads to gain-of-function activation of PI3Kα manifested by increased lipid kinase activity, growth-factor independent activation of Akt signaling, cellular transformation, and the generation of tumors in a diverse array of preclinical models (10–14).

The strong evidence underscoring the oncogenic nature and the high frequency of PIK3CA mutations raise the hypothesis that selective inhibition of PI3Kα may have robust antitumor efficacy in the PIK3CA-mutant cancer population. In addition, a selective agent may offer the opportunity to spare patients the side-effects associated with broader inhibition of the class I PI3K family. Based on this notion, we sought to develop a PI3Kα-selective inhibitor to allow the testing of this therapeutic hypothesis in man.

Here we report the biologic properties of the 2-aminothiazole derivative NVP-BYL719. The compound is a selective PI3Kα inhibitor equipotent against the wild type and the most common somatic mutations of PI3Kα. NVP-BYL719 has excellent drug-like properties and in vivo administration of NVP-BYL719 results in significant dose-dependent antitumor efficacy in mice bearing PIK3CA-dependent tumor xenograft models as well as an improved safety profile with respect to glucose metabolism when compared with pan-PI3K inhibition. To refine the design of clinical trials and identify potential predictive biomarkers for patient selection, NVP-BYL719 was profiled across a large panel of cancer cell lines referred to as the Cancer Cell Line Encyclopedia (CCLE; ref. 15). The analysis of the underlying genetic aberrations driving NVP-BYL719 sensitivity revealed PIK3CA mutation as the foremost positive predictor of sensitivity as well as additional positive and negative associations such as PIK3CA amplification and PTEN mutation, respectively. NVP-BYL719 predictive features were found to be relevant for predicting response in vivo in diverse cancer cell line-derived and patient-derived xenograft models tested in mice. Using a novel analytical approach to define the selectivity index of small molecule inhibitors across the CCLE, NVP-BYL719 showed markedly selective efficacy in PIK3CA mutants when compared with wild-type cell lines and when compared with pan-PI3K inhibitors. These data provided the rationale for conducting the initial clinical testing of NVP-BYL719 exclusively in preselected patients with advanced solid malignancies carrying PIK3CA gene alterations (16).

Materials and Methods

Chemical entities

NVP-BYL719 and NVP-BKM120 were synthesized by Global Discovery Chemistry Department (NIBR, Novartis, Basel, Switzerland). For in vitro studies, 10 mM stock solutions were prepared in 100% dimethyl sulfoxide. For in vivo experiments, NVP-BYL719 was formulated for oral administration in solution by solving the compound in N-methyl pyrrolidine, polyethylene glycol 300, solutol HS15, and water (10%:30%:20%:40%, v/v) or in suspension in 1% (w/v) carboxymethylcellulose (CMC) + 0.5% (w/v) Tween 80 similar to NVP-BKM120.

Antibodies

Antibodies used for Western blot analysis, reverse phase protein array (RPFA) and in cell Western assays were p-Akt (S473) (#9271), total Akt (#9272), p-IRP56 (#2211), total RPS6 (#2317), p-p53 (S15) (#9284), and p-ATM (S1981) (#4526) from Cell Signaling and anti-tubulin β from Thermo Fisher.

Cell lines and in vitro compound profiling

Mechanistic models. To evaluate the isoform-specific potency of NVP-BYL719 in a cell-based system, an N-terminally myristoylated form of each PI3K class IA isoform was expressed in Rat1 fibroblasts as described in ref. 17. The retroviral expression plasmid pBabePuro containing human p110α, p110β, and p110δ with an N-terminal myristoylation (myr) signal followed by an HA-tag were generated. Successfully infected Rat1 cells were selected in medium containing 4 μg/mL of puromycin, expanded and characterized for expression of the p110 isoforms (in 2006). Transgenic expression of the myristoylated protein was confirmed by increased levels of phosphorylated Akt. The TSC1+/−; null MEFs mechanistic model for mTORC1 constitutive activation has been obtained from Dr. D. Kwiatkowski in 2007 (Brigham and Women’s Hospital, Boston, MA).

Human tumor cell lines. The A549 (American Type Culture Collection, ATCC) and the U2OS (ATCC) were authenticated by single-nucleotide polymorphism (SNP) analysis in 2006. The biochemical kinase assays and RPFA assays were conducted as previously described (17, 18).

High-throughput pharmacologic cell line profiling

Cell lines obtained from ATCC, DSMZ, and HSSRB are cultured in RPMI or Dulbecco’s modified Eagle medium plus 10% FBS (Invitrogen) at 37°C, 5% CO2 using automated processing. Cell line identities were confirmed using a 48 variant SNP panel comparing the previous cell line tests as mentioned in ref. 19. A detailed description of the cell lines and of the high-throughput cell viability assays can be found in ref. 15, see also http://www.broadinstitute.org/ccle.

In vivo studies in mice and rats

All animal studies were conducted in accordance with protocols approved by the Novartis Institutes for BioMedical Research Animal Care and Use Committee.

Cell lines-derived tumor models. All in life experimentation and efficacy studies were conducted as described previously (17). Tumor xenografts were grown subcutaneously or orthotopically in nude mice (Harlan, Germany) or nude Rowett rats (Hsd: R1-Fox1nu, Harlan, The Netherlands) by injection of 3 × 10^6 to 1 × 10^7 cells.
or implantation of tumor fragments of approximately 50 mg. Tumor-bearing animals were treated with either vehicle control, NVP-BYL719, or NVP-BKM120 (p.o., every day) at the doses indicated.

**Patient-derived tumor models.** Patient-derived xenograft (PDX) models were established by implanting surgical tumor tissues from treatment-naïve cancer patients into nude mice. All samples were anonymized and obtained with informed consent and under the approval of the institutional review boards of the tissue providers and Novartis. All PDX models were histologically characterized and external diagnosis was independently confirmed by in-house pathologists and were genetically profiled using various technology platforms after serial passages in mice. PIK3CA mutation was determined by both RNA and DNA deep sequencing technologies and PIK3CA amplification was determined by SNP array 6.0. For efficacy studies, tumor-bearing animals were enrolled when subcutaneously implanted tumors reached about 200 mm³ and treated with NVP-BYL719 at 50 mg/kg daily. The response is reported as percentage change in tumor volume at last day of treatment relative to day 0 (start of treatment).

**Automated calls of NVP-BYL719 sensitivity**

Starting from the vector of responses \( A_{\text{max}} \) or \( EC_{50} \), we considered the shape of the rank-ordered plot of response values (for \( A_{\text{max}} \), log-transformed \( EC_{50} \)) in order to assign cell lines into responder, intermediate, and nonresponder classes using a combination of \( EC_{50} \) and \( A_{\text{max}} \) cutoffs defined with this method.

**Genetic and genomic characterization of cell lines**

A detailed description of DNA copy number and mutation data can be found in ref. 15, see also http://www.broadinstitute.org/ccle. In addition, only dominant negative or functional mutations identified through manual literature curation were considered in the analyses. Wild types were those with no identified sequence variants.

**NVP-BYL719 compound differential selectivity analysis**

To assess NVP-BYL719 selectivity on PIK3CA mutants, an internal panel of drug compounds was profiled across both the PIK3CA mutant and wild-type cell lines. For each compound, the response profiles (\( A_{\text{max}} \) values) of the cell lines were converted to a selectivity score by multiplying the z-score \( z = \frac{A_{\text{max}}}{\text{compound}} - \frac{A_{\text{max}}}{\text{wild-type}} \) to the compound were z-transformed. The z-scores of the mutant responses were then compared against those of the wild-type lines by taking the difference between the 2 and then back-converting to a \( P \) value. The \( P \) values across the different compounds were then corrected for multiple hypotheses testing using the Benjamini–Hochberg false discovery rate (FDR) method (20).

**Results**

**NVP-BYL719 potently and selectively inhibits PI3K \( \alpha \) in vitro**

PI3K, \( \beta \), \( \delta \), and \( \gamma \) enzymes share significant amino acid residue homology with particularly high conservation in the catalytic kinase domain. The 2-aminohiazole scaffold was selected as a starting point for the development of potent and selective PI3K inhibitors based on its binding mode, indicating the potential to use substituents at the amino group to develop interactions with nonconserved amino acids at the ATP pocket entrance (21). Consequently, systematic modification of key moieties and optimization of the drug-like properties led to the identification of NVP-BYL719 (18).

As previously described in ref. 18, in biochemical assays NVP-BYL719 inhibits wild-type PI3K\( \alpha \) (IC\( _{50} \) = 4.6 nmol/L) more potently than the PI3K\( \delta \) (IC\( _{50} \) = 290 nmol/L) and PI3K\( \gamma \) (IC\( _{50} \) = 250 nmol/L) isoforms and shows significantly reduced activity against PI3K\( \beta \) (IC\( _{50} \) = 1,156 nmol/L). Here, in addition, we show that NVP-BYL719 potently inhibits the 2 most common PIK3CA somatic mutations (H1047R, E545K; IC\( _{50} \) \( \leq \) 290 nmol/L) and the compound also lacked activity against the class III family member Vps34 and the related class IV PIKK protein kinases mTOR, DNA-PK, and ATR and was significantly less potent against the distinct lipid kinase PIK4B (Table 1).

The kinase selectivity profile of NVP-BYL719 was further examined in *in vitro* kinase assay panels. Among all the kinases tested (excluding class I PI3K and PIK4B) their respective IC\( _{50} \) or Kd values were at least 50-fold higher.

**Table 1. Effects of NVP-BYL719 against PI3K lipid or protein kinases**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>PI3K( \alpha )</th>
<th>E54K mutant</th>
<th>H1047R mutant</th>
<th>PI3K( \delta )</th>
<th>PI3K( \gamma )</th>
<th>PI3K( \beta )</th>
<th>PI3K( \delta )</th>
<th>PI3K( \beta )</th>
<th>mTOR</th>
<th>DNA-PK</th>
<th>ATR</th>
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<td>KG</td>
<td>KG</td>
<td>TR-FRET</td>
<td>Caliper</td>
<td>u-screen</td>
</tr>
<tr>
<td>BYL719</td>
<td>4.6 ± 0.4</td>
<td>4.0 ± 0.6</td>
<td>4.8 ± 0.4</td>
<td>1.156 ± 77</td>
<td>290 ± 180</td>
<td>250 ± 140</td>
<td>581 ± 42</td>
<td>&gt;9,100</td>
<td>&gt;9,100</td>
<td>&gt;9,100</td>
<td>&gt;15,000</td>
</tr>
</tbody>
</table>

IC\( _{50} \), nmol/L.

**NOTE:** *In vitro* assays were conducted with the indicated recombinant PI3K lipid or protein kinases in the presence of increasing concentrations of NVP-BYL719 as described in ref. 17. The concentration producing 50% inhibition of the enzymatic activity (IC\( _{50} \)) in nmol/L as determined in multiple experiments (\( n = 2-20 \)) is shown as an average ± SD. Abbreviation: KG, KinaseGlo.
Figure 1. PK/PD/efficacy relationship of NVP-BYL719 in PI3Kα-dependent tumor mouse models in vivo. A, female athymic mice bearing subcutaneous xenotransplants of Rat1-myr-p110α tumors were treated with single or repeated doses of 12.5, 25, and 50 mg/kg, p.o. At the indicated time points, the groups of mice (n = 2–15 per group) were sacrificed and blood and tissues were collected. Each tumor tissue was flash frozen, then pulverized and analyzed by RPPA to determine S473P-Akt levels and, in parallel, the concentration of NVP-BYL719 was quantified. PK/PD relationship for each dose level was established by comparing % of inhibition of S473P-Akt levels versus control with NVP-BYL719 concentration at each time point indicated (*, P < 0.05). Results are presented as mean ± SEM. B, female athymic mice bearing Rat1-myr-p110α subcutaneous xenografts were treated with vehicle (red dots) or NVP-BYL719 at 12.5 (green triangles) mg/kg/day p.o., 25 (red squares) mg/kg/day p.o., and 50 (orange diamonds) mg/kg/day p.o., every day (n = 8 per group), respectively. (Continued on following page.)
cally significant antitumor effect with a T/C of 14.1% and tolerated and resulted in a dose-dependent and statisti-
(Fig. 1B). Treatments of 12.5, 25, and 50 mg/kg were well every day with the compound for up to 8 consecutive days
way inhibition was linked to antitumor activity, Rat1-
pathway, which notably paralleled time-dependent drug
dose and time-dependent inhibition of the PI3K/Akt
were collected for PK and PD analysis at different time
25, or 50 mg/kg, p.o.) and plasma and tumors samples
received single or repeated doses of NVP-BYL719 (12.5,
tumor-bearing mouse model. Each female athymic mouse
relationships were assessed in a Rat1-myr-p110
in vivo
- driven tumors
involved in DNA-damage repair (ATM and ATR) pro-
NVP-BYL719 does not seem to interfere with the PIKKs
involved in DNA-damage repair (ATM and ATR) pro-
Together, these data strongly support the notion that NVP-BYL719 has the relevant in vivo properties of a selective PI3Kα inhibitor.
NVP-BYL719 shows robust PK/PD/Efficacy relationship in PI3Kα-driven tumors
To examine NVP-BYL719 ability to inhibit the PI3K/ Akt pathway in a PI3Kα-dependent in vivo model, its pharmacokinetic/pharmacodynamic (PK/PD) relationship was assessed in a Rat1-myr-p110α mechanistic tumor-bearing mouse model. Each female athymic mouse received single or repeated doses of NVP-BYL719 (12.5, 25, or 50 mg/kg, p.o.) and plasma and tumors samples were collected for PK and PD analysis at different time points. Here NVP-BYL719 treatment was associated with dose and time-dependent inhibition of the PI3K/Akt pathway, which notably paralleled time-dependent drug exposure in tumor and plasma (Fig. 1A).

To determine whether dose- and time-dependent pathway inhibition was linked to antitumor activity, Rat1-myr-p110α tumor-bearing nude mice were treated orally every day with the compound for up to 8 consecutive days (Fig. 1B). Treatments of 12.5, 25, and 50 mg/kg were well tolerated and resulted in a dose-dependent and statistically significant antitumor effect with a T/C of 14.1% and regressions of 9.6% and 65.2%, respectively. To assess the relative PI3K selectivity in vivo, we further tested NVP-
BYL719 in a corresponding Rat1-myr-p110β model. NVP-
BYL719, when tested at the optimal dose of 50 mg/kg p.o., every day, showed only a modest antitumor effect (T/C of 30%; Fig. 1C).

We next sought to better understand the degree of PI3Kα inhibition that is required for antitumor efficacy. To this end, we first determined the tumor concentrations giving 50% (in vivo $IC_{50}$) and 80% (in vivo $IC_{80}$) S473P-Akt inhibition (0.4 and 4 μmol/L, respectively) by measuring the extent of Akt phosphorylation using RPPA and the specific tumor drug concentration in matched samples from multiple animals and at multiple time points (Fig. 1D). Interestingly, when corrected for plasma protein binding of NVP-BYL719 in mouse (PPB = 91.2%), the in vivo $IC_{50}$ (35 nmol/L) and $IC_{80}$ (352 nmol/L) values roughly approximate the in vitro cellular $IC_{50}$ and $IC_{80}$ of 74 and 301 nmol/L, respectively. We next sought to determine the relationship between exposure, as measured by time over the in vivo $IC_{80}$, and antitumor efficacy. Here, we found a nearly linear relationship between the antitumor efficacy magnitude and duration of drug exposure over the $IC_{80}$ ($R^2 = 0.80$, $P < 0.001$, $n = 11$; Fig. 1E). From this relationship it seems that 80% inhibition of Akt phosphorylation for at least 29% of the dosing interval is required for NVP-BYL719 to induce tumor stasis, and that this level of pathway inhibition must be sustained for at least 45% of the dosing interval to produce 30% tumor regression in the Rat1-myr-p110α tumor-bearing nude mice. In contrast, in the Rat1-myr-p110β tumor-bearing nude mice NVP-BYL719 exposure levels did not achieve 80% inhibition of Akt phosphorylation (in vivo $IC_{50}$ = 29 μmol/L; corrected for NVP-BYL719 plasma protein binding in mouse $IC_{80}$ = 2,552 μmol/L) most likely explaining the modest antitumor effect observed and in line with the modest activity of the compound on p110β. To exclude the possibility that our finding could be Rat1 mouse tumor models specific, NVP-BYL719 was administered in vivo at different doses to nude mice and nude rats bearing a diverse range of cancer cell lines–derived tumor xenografts. Here as well, we found a nearly linear relationship between the antitumor efficacy magnitude and duration of drug exposure over the $IC_{80}$ ($R^2 = 0.77$, $P < 0.001$, $n = 27$, Supplementary Fig. S4 and Table S5). These data suggest that sustained inhibition of the PI3K/Akt pathway for a fraction of the dosing interval is required for NVP-BYL719 to produce a robust anti-tumor effect.
NVP-BYL719 shows an improved safety profile compared with pan-class I inhibition

The expected on target side effects of anti-PI3K therapy are insulin resistance and hyperglycemia. To assess whether NVP-BYL719 perturbs glucose homeostasis, plasma insulin and glucose blood levels were measured and compared with plasma drug concentrations in matched samples from multiple animals and at multiple time points. The data here revealed that insulin plasma levels increased proportionally with NVP-BYL719 plasma concentrations, whereas blood glucose levels were maintained close to normal up to 20 μmol/L of NVP-BYL719 (Fig. 2A and B). However, above 20 μmol/L, we observed a compound concentration-dependent glucose increase which led to hyperglycemia despite insulin plasma level elevation. Thus, we defined 20 μmol/L as NVP-BYL719-related hyperglycemic threshold in mice.

We next hypothesized that the body weight loss we observed following compound treatment might correlate with the severity of hyperglycemia. In keeping with this notion, we observed a nearly linear relationship between the body weight loss magnitude and duration of exposure above NVP-BYL719 hyperglycemia threshold (20 μmol/L; R² = 0.90; P < 0.001; n = 11; Fig. 2C). From this relationship, it seems that the compound exposure levels should be sustained for no more than 36% of the dosing interval above the hyperglycemia cutoff to maintain body weight loss below 5% in mice.

To determine the therapeutic index of NVP-BYL719 with respect to glucose homeostasis, we next compared the dose estimated to produce 30% tumor regression (20 mg/kg) based on the duration of exposure above the IC₈₀ threshold for S473P-Akt inhibition with the dose estimated to induce 5% body weight loss (65 mg/kg) based on the duration of exposure above the hyperglycemia threshold. We estimated a therapeutic index value of 3.25 for NVP-BYL719 (Fig. 2D). A similar analysis was conducted with the pan-PI3K inhibitor NVP-BKM120 (17), leading to a therapeutic index value of 1.1 (Fig. 2E). These data suggest that PI3Kα-selective agents such as NVP-BYL719 may impact physiologic pathways such as glucose metabolism differentially from a broader inhibition of the class I PI3K family.

**PI3KCA amplification and PTEN mutation also modulate response to NVP-BYL719**

The CCLE profiling results confirmed that PI3KCA mutation status affected NVP-BYL719 sensitivity, but also suggested the importance of additional, modulating factors. Among the PI3KCA mutant cell lines, 6 were nonresponders to NVP-BYL719, whereas among the PI3KCA wild-type cell lines, 100 were responders. Hence, PI3KCA mutation status enriches for but does not uniquely explain NVP-BYL719 response. We consequently examined the degree of association between the genetic status of other genes to NVP-BYL719 response, including PTEN, KRAS, NRAS, and BRAF mutation, as well as ERBB2 and PI3KCA amplification (Fig. 4A). In the overall cell lines population, similar to PI3KCA mutation (P value of $7.5 \times 10^{-5}$, FDR of $1.8 \times 10^{-5}$), PI3KCA amplification, and NRAS mutation were identified to be positively associated with NVP-BYL719 response (P value of 0.0017, FDR of 0.109 and P value of 0.011, FDR of 0.147, respectively). ERBB2 amplification showed a trend to be associated with NVP-BYL719 response and BRAF mutation was rather associated with nonresponse; however, both features just missed the FDR < 0.25 cutoff. KRAS mutation and PTEN mutation, as independent genetic features, were close to neutral. Considering that the pivotal feature for patient stratification in the clinic will be mutant PI3KCA, we performed a more detailed examination of predictive features across the PI3KCA mutant cell line population versus wild type, with

Based on those 2 criteria (EC₅₀ and Aₘₐₓ), 122 cell lines were categorized as responder (Supplementary Table S6), 118 were categorized as intermediate and 234 were categorized as nonresponder to NVP-BYL719 treatment (Fig. 3A). We next asked whether any commonly found genetic or pathway aberrations were associated with NVP-BYL719 in vitro responses. PIK3CA mutation is the first feature we examined based on the underlying genetic hypothesis for developing a selective PI3Kα inhibitor (Fig. 3B). Interestingly, we found that cell lines that carry PIK3CA mutation are more likely to be NVP-BYL719 responsive, with 22 responder cell lines out of total of 34, or 64% response rate, significantly higher than the 100 of 440 found in PIK3CA wild-type group, or 22% response rate. Hence, the PIK3CA mutation feature alone should allow for a 3-fold improvement in response rate versus PIK3CA wild type and a 2.5-fold over random (as overall population has a 25% response rate). Strikingly, using an unbiased approach, PIK3CA mutation was found to be the most significant mutation feature that predicts NVP-BYL719 response among the 25 mutation features (restricted to known functional mutations for all genes tested) included in the analysis (Fig. 3C). A close-up examination of the lineage distribution among PIK3CA mutants indicated that the preferred sensitivity to NVP-BYL719 can be observed in almost all lineages, suggesting that the genetic status should represent a reliable patient enrollment criterion across indications (Supplementary Table S6).
Figure 2. Determination of NVP-BYL719 safety profile compared with pan-class I PI3K inhibitors. A, relationship between plasma insulin levels and plasma NVP-BYL719 concentrations measured in the same probe following NVP-BYL719 treatment. B, relationship between blood glucose levels and plasma NVP-BYL719 concentrations measured in the same probe following NVP-BYL719 treatment. The in vivo hyperglycemia threshold for NVP-BYL719 (20 μmol/L) is represented by a dotted line. C, linear correlation observed between the fraction of time over plasma hyperglycemia threshold (20 μmol/L) between two consecutive dosings and body weight loss in the Rat1-myr-P110α tumors (gray dots) and Rat1-myr-p110δ tumors (black dots); R² = 0.90, P < 0.001, n = 11. D, efficacy curve (gray dots) as determined by the fraction of time above the IC₈₀ threshold for S473P-Akt and tolerability curve (orange dots) as determined by the duration of exposure above NVP-BYL719 hyperglycemia threshold (20 μmol/L) in mice treated orally every day with increasing doses of NVP-BYL719 up to 75 mg/kg. E, efficacy curve (gray dots) as determined by the fraction of time above the IC₈₀ threshold for S473P-Akt and tolerability curve (orange dots) as determined by the duration of exposure above NVP-BKM120 hyperglycemia threshold (6 μmol/L) in mice treated orally every day with increasing doses of NVP-BKM120 up to 60 mg/kg.
the aim of further refining the selection and enhancing response rate. This analysis indicated that PTEN mutation is associated with nonresponse (P value of 0.022, FDR of 0.22) in the mutant PIK3CA context only, whereas PIK3CA amplification is positively associated with NVP-BYL719 sensitivity in the PIK3CA wild-type population only (P value of 0.0037 and FDR of 0.22). Following these findings, we constructed a hypothesis-based predictor of response to improve upon the single feature model based on PIK3CA mutation status, by combining PIK3CA amplification and PTEN mutation as significant predictive features in the respective settings (Fig. 4B). This combined genetic predictor is significantly associated with NVP-BYL719 responders versus nonresponders (P value = 1.49 × 10−7). Cross-validation using bootstrapping shows that the predictor significantly enriched for responders (positive predictive value = 76%). However, this predictor has a sensitivity of only 21%, which means that it missed ~80% of the responders. Future work needs to be focused on identifying features that predict sensitivity in this remaining 80% of the responders not explained by PIK3CA mutation or amplification.

Genetic alterations in PIK3CA predict NVP-BYL719 in vivo efficacy

Next, NVP-BYL719 was administered in vivo at the dose of 50 mg/kg (every day, p.o.) to mice bearing a diverse range of cancer cell lines–derived tumor xenografts (Fig. 5A and Supplementary Table S5) with different genetic backgrounds, including the predictive features of the decision tree described previously. Most of the tumor models that carried a PIK3CA mutation or amplification responded to NVP-BYL719 (response defined as T/C > 20%). In contrast, in most of the tumor models that carried a PTEN mutation or were PIK3CA wild type, we observed progressive disease. In vivo, the predictor also significantly enriched for responders (positive predictive value = 89%). These data demonstrate that the NVP-BYL719 predictive features derived from the in vitro profiling and analysis of the CCLE seem relevant for predicting response in vivo (P = 0.01, Fisher test).

Considering that PIK3CA mutation or amplification might be the key molecular determinants for NVP-BYL719 patient stratification in the clinic, we next performed a molecularly defined prospective trial in PIK3CA mutant and/or amplified PDX models in mice with the aim to test our patient selection strategy in a setting that best mimics disease response in patients. Tumor-bearing animals were treated with NVP-BYL719 at 50 mg/kg/day for 14 to 16 days. Strikingly, 8 of 9 PDX models that carry a mutation and/or amplification in PIK3CA responded to NVP-BYL719, leading to a response rate of 88% (Fig. 5B and Supplementary Table S7). The PDX response to NVP-BYL719 can be observed in different lineages (breast, lung, gastric, colorectal cancer), suggesting that PIK3CA genetic status should represent a reliable patient enrollment criterion across indications.
PIK3CA mutant cell lines are selectively sensitive to NVP-BYL719

The above-mentioned approach was useful in defining what tumors were responsive to PI3Kα inhibition. An independent question is asked when one considers which therapeutic modality is most selective and hence likely to have the best therapeutic index in a specific cancer genotype. Here, using a novel analytical approach to define the selectivity index of small molecule inhibitors across the CCLE, we compared the selectivity profiles across different compound treatments (~1,000) encompassing more than 200 mechanisms of actions in PIK3CA mutant versus wild-type cell lines and ranked the compounds based on the magnitude of their effects in these 2 groups (Fig. 6). NVP-BYL719, together with 3 close analogs, showed markedly selective efficacy in PIK3CA mutants when compared with wild-type cell line populations and when compared with pan-PI3K inhibitors. Conversely, MEK inhibitors were differentially more selectively effective in PIK3CA wild-type cell lines compared with mutants.

Discussion

The genes comprising the PI3K pathway are commonly altered in human cancer and targeting this pathway represents an important area for therapeutic development. Indeed, many agents targeting diverse nodes in the pathway are currently in clinical trials. However, most of these compounds are not selective for PI3Kα and inhibit other PI3K isoforms and/or other downstream nodes. Several reported phase I study results for pan-PI3K inhibitors showed that pharmacological inhibition of PI3K in humans is feasible. Preliminary evidence of antitumor
activity in patients with solid tumors has been observed and the associated adverse events indicate on-target toxicity such as hyperglycemia (22, 23). However, as clinical trials with PI3K inhibitors have so far been conducted in un-preselected patients, the patient response rate and its extent seem more modest in comparison to other targeted agents such as BRAF inhibitors in BRAF-mutated melanoma (24) or crizotinib in ALK-translocated tumors (25). These data raise 2 related key questions as reviewed in ref. 26: could the efficacy observed in the clinic be limited by the safety profile of pan-class I isoforms inhibition and could the identification of the patient populations that are likely to benefit the most from the treatment lead to more frequent and pronounced antitumor effects in humans similar to what has been reported preclinically?

The discovery of somatic PIK3CA missense mutations and their frequency in a number of common solid tumors raise the possibility that PI3Kα-selective inhibitors might be safer and specifically efficacious in preselected PIK3CA-mutant patients compared with
pan-class I inhibitors. In this study, we reported that NVP-BYL719 potently inhibits the PI3Kα isoform and its 2 most common oncogenic mutants and is selective against the other class I PI3K isoforms and a wide range of other kinases. The compound activity and selectivity profile combined with excellent drug-like properties translated in vivo, in robust dose- and time-dependent inhibition of PI3Kα signaling, resulting in good therapeutic efficacy against PIK3CA-dependent tumors. These results showed that selective inhibition of PI3Kα lead to robust efficacy in PIK3CA-dependent tumor models comparable to pan-class I inhibitors as previously reported (17, 27). One additional pending question is whether PI3Kα inhibitors require a high and/or continuous inhibition of the PI3K/Akt pathway to produce a robust antitumor activity. We showed through a detailed PK/PD/efficacy relationship analysis that the fraction of time above the S473P-Akt IC80 value is a key determinant associated with NVP-BYL719 efficacy in vivo, suggesting that sustained inhibition of the pathway for a fraction of the time period between 2 consecutive dosing is likely needed.

As PI3Kα-selective inhibitors have been developed very recently, there is only minimal information available about on the safety when compared with broader PI3K inhibition. Of special concern with PI3Kα inhibitors is the induction of insulin resistance as the PI3K pathway plays a predominant role in glucose homeostasis, and hyperglycemia has been reported to be one of the most frequent adverse events in clinical trials. Jensen and colleagues have recently demonstrated that PI3Kα-selective inhibition did not perturb glucose homeostasis in rodents in contrast to pan-class I inhibition (28). In our study, by conducting a more detailed PK/PD/efficacy/tolerability relationship analysis we could provide robust evidence that NVP-BYL719 has a better therapeutic window compared with pan-class I inhibition (53-fold shift), in line with our working hypothesis.

As mentioned previously, one other major challenge in the clinical development of PI3K inhibitors is to identify patient populations who will most likely benefit from the treatment. Thus, active efforts are currently made to better define patient stratification methods to maximize therapeutic responses to such “personalized” therapies. Some preclinical studies have already found association with activating PIK3CA mutations and response to pan-PI3K inhibitors (17, 29, 30). However, one study conducted with different PI3K inhibitors in a panel of 39 cell lines from 9 distinct lineages did not show enhanced activity in PIK3CA mutant lines (31). Here we report for the first time a large-scale analysis of sensitivity to a PI3Kα-selective inhibitor using the recently established CCLE (15). The integration of gene expression, chromosomal copy number, and massive parallel sequencing data from ~1,000 human cancer cell lines with pharmacologic profiles for anticancer drugs allows the interrogation of the dataset toward the identification of genetic, lineage, and gene-expression–based predictors of drug sensitivity. In our study, the comprehensive analysis has revealed multiple factors that are capable of predicting NVP-BYL719 sensitivity. Hence, we found that PIK3CA mutation is not only associated with NVP-BYL719 response, but is the most significant mutation feature that could predict NVP-BYL719 sensitivity among 25 mutation features included in the unbiased analysis of such large scale dataset. The preferred sensitivity to NVP-BYL719 among PIK3CA mutants can be observed in almost all lineages, suggesting that the genetic status should represent a reliable patient enrollment criterion across indications. To confirm that PI3Kα inhibition is the best way to effectively and selectively target the PIK3CA-mutant cancer cell population compared with the wild-type, we also compared the response profiles of PIK3CA mutant versus wild-type cell lines across different compound treatments (~1,000) encompassing more than 200 MoAs and ranked the compounds based on the magnitude of their effects in the 2 groups. In general, PI3Kα inhibitors were selectively effective in PIK3Cα mutant cells when compared with wild type, with NVP-BYL719 being among the most selective and ranking higher than pan-PI3K inhibition.

In addition to PIK3CA mutation, we also found PIK3CA amplification to be positively associated with...
NVP-BYL719 sensitivity in the overall and interestingly also in the \textit{PIK3CA} WT cell line population. \textit{NRAS} mutation correlated with higher drug sensitivity within the entire cell lines tested or within the \textit{PIK3CA} WT cell line population although here it just missed the FDR < 0.25 cutoff. This is to our knowledge, the first report that revealed \textit{NRAS} mutation being associated with PI3K\(\alpha\) selective inhibition.

The identification of the patient population that would most benefit from PI3K inhibitors such as NVP-BYL719 not only requires the determination of positive predictors but similarly of molecular features that are associated with primary resistance. Interestingly, we observed a negative association of \textit{PTEN} mutation in the \textit{PIK3CA}-mutated population only. This result seems to contrast with a previously reported study where cell lines harboring double alterations in \textit{PIK3CA} and \textit{PTEN} were significantly more sensitive to the pan-class I PI3K inhibitor GDC0941 than cell lines with no detectable alterations in the signaling pathway (29). However, it has been demonstrated in preclinical models that \textit{PTEN}-deficient tumors are more dependent on PI3K\(\beta\) signaling than on PI3K\(\alpha\) (31–34), hence, treatment of dual \textit{PIK3CA}- and \textit{PTEN}-mutated cancers might require inhibitors with activity against both PI3K\(\alpha\) and PI3K\(\beta\) such as pan-class I PI3K inhibitors. In contrast to previously reported results (35), concurrent \textit{KRAS} mutation did not confer resistance to NVP-BYL719 in \textit{PIK3CA}-mutated cell lines. Some \textit{KRAS} mutations preferentially signal through the PI3K pathway; however, \textit{KRAS} mutation has also been described as a resistant factor for PI3K inhibitors (12, 36) and this opposite interplay may be context or lineage specific.

Importantly, the hypothesis-based predictor we have developed by combining \textit{PIK3CA} mutations with other significant features, \textit{PIK3CA} amplification and \textit{PTEN} mutation showed an improved PPV of 76\% and translated \textit{in vivo} across a selection of cancer cell lines as well as PDX tumor xenograft mouse models. However, this predictor, with a sensitivity of 21\%, missed ~80\% of the responders that are not associated with the selected 3 features, indicating that other predictive markers of NVP-BYL719 response may be very context specific and may require further investigation of selected subgroup of cell lines or indications that may not be represented in sufficient numbers to allow a statistically significant score in the current dataset.

Based on the results obtained so far demonstrating that NVP-BYL719 is a selective PI3K\(\alpha\) inhibitor with good drug-like and pharmacologic properties and that genetic alterations in \textit{PIK3CA} is the most significant predictive feature of selective sensitivity to the compound, NVP-BYL719 has been the first PI3K\(\alpha\)-selective inhibitor to enter phase I clinical development in preselected adult patients with advanced solid malignancies carrying \textit{PIK3CA} gene alterations. Preliminary clinical data available indicate that NVP-BYL719 is well tolerated, with manageable side effects, a predictable PK profile and some objective responses and prolonged disease stabilization with tumor shrinkage have already been reported (16, 37). These encouraging first clinical results suggest that the validity of the hypothesis, the quality of the molecule, and the patient selection criteria identified and assessed preclinically are likely to translate in patient benefit. We therefore aim at integrating current and to be discovered patient selection criteria in the design of the future-clinical trials with the objective of enhancing response rate and benefit for the patients bearing tumors with the highest likelihood of being sensitive to NVP-BYL719.

\section*{Disclosure of Potential Conflicts of Interest}

M. Boehn has ownership interest (including patents) in Novartis Pharma AG. C. Garcia-Echeverria has ownership interest (including patents) in Sanofi. R. Schlegel has ownership interest (including patents) in Novartis Pharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

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