Drugs of the Future 2013, 38(2): 73-79

CCC: 0377-8282/2013
DOI: 10.1358/dof.2013.38.2.1924783

BUPARLISIB

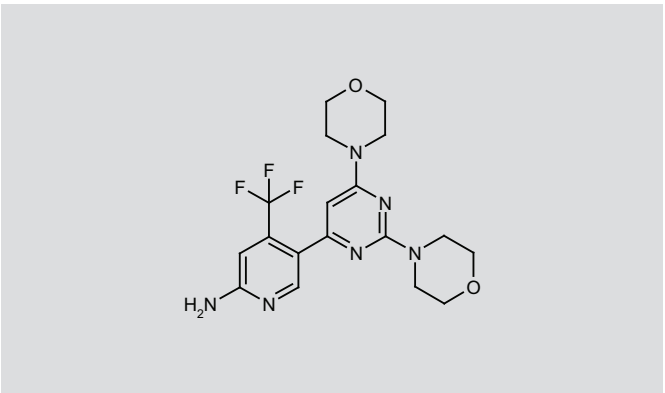
Rec INN

Phosphatidylinositol 3-Kinase Alpha Inhibitor
Oncolytic

NVP-BKM-120

5-[2,6-Di(morpholin-4-yl)pyrimidin-4-yl]-4-(trifluoromethyl)pyridin-2-amine

InChI: 1S/C18H21F3N6O2/c19-18(20,21)13-9-15(22)23-11-12(13)14-10-16(26-1-5-28-6-2-26)25-17(24-14)27-3-7-29-8-4-27/h9-11H,1-8H2,(H2,22,23)



$C_{18}H_{21}F_3N_6O_2$
Mol wt: 410.3935
CAS: 944396-07-0
EN: 458123

SUMMARY

The PI3K signaling pathway is dysregulated in many tumor types, where it is associated with a poor prognosis and resistance to multiple therapies, and inhibition of PI3K is an emerging strategy in the treatment of cancer. Buparlisib (NVP-BKM-120) is a 2,6-dimorpholinopyrimidine derivative and a potent pan-PI3K inhibitor, inhibiting PI3K downstream signaling, including downregulation of p-Akt and p-S6R, and inducing apoptosis of cancer cells. In animal models, buparlisib inhibited tumor growth and metastasis of breast, lung, glioma, multiple myeloma, gastric and soft tissue sarcoma, and it synergized with other cytotoxic agents. Buparlisib is rapidly absorbed and highly bioavailable after oral administration, with a half-life of approximately 40 hours, mainly via hepatic clearance. The product is currently being tested in clinical trials in several solid tumors and was found to be well tolerated, preliminary data demonstrating variable clinical responses including partial response, stable disease and progressive disease.

Key words: PI3K signaling pathway – Treatment of cancer – Pan-PI3K inhibitor – Buparlisib – NVP-BKM-120

SYNTHESIS*

Buparlisib can be prepared by two related ways:

Cyclization of morpholine-4-carboximidamide hydrobromide (I) with diethyl malonate (II) by means of NaOEt in refluxing EtOH gives 2-(morpholin-4-yl)pyrimidine-4,6-diol (III), which by chlorination with $POCl_3$ at 120 °C affords 4,6-dichloro-2-(morpholin-4-yl)pyrimidine (IV). Nucleophilic substitution of the dichloropyrimidine (IV) with morpholine (V) in the presence of Et_3N in refluxing *N*-methyl-2-pyrrolidone provides 2,4-di(morpholin-4-yl)-6-chloropyrimidine (VI) (1). Alternatively, dimorpholinopyrimidine (VI) is prepared by reaction of 2,4,6-trichloropyrimidine (VII) with morpholine (V) in THF, followed by chromatographic separation (1-3). Finally, chloropyrimidine derivative (VI) is submitted to a Suzuki cross-coupling reaction with boronate ester (VIII) in the presence of $Pd(dppf)_2Cl_2·CH_2Cl_2$ (2) or $Pd(dppf)_2Cl_2$ (1) and Na_2CO_3 in 1,2-dimethoxyethane at 90-95 °C (1, 2). Scheme 1.

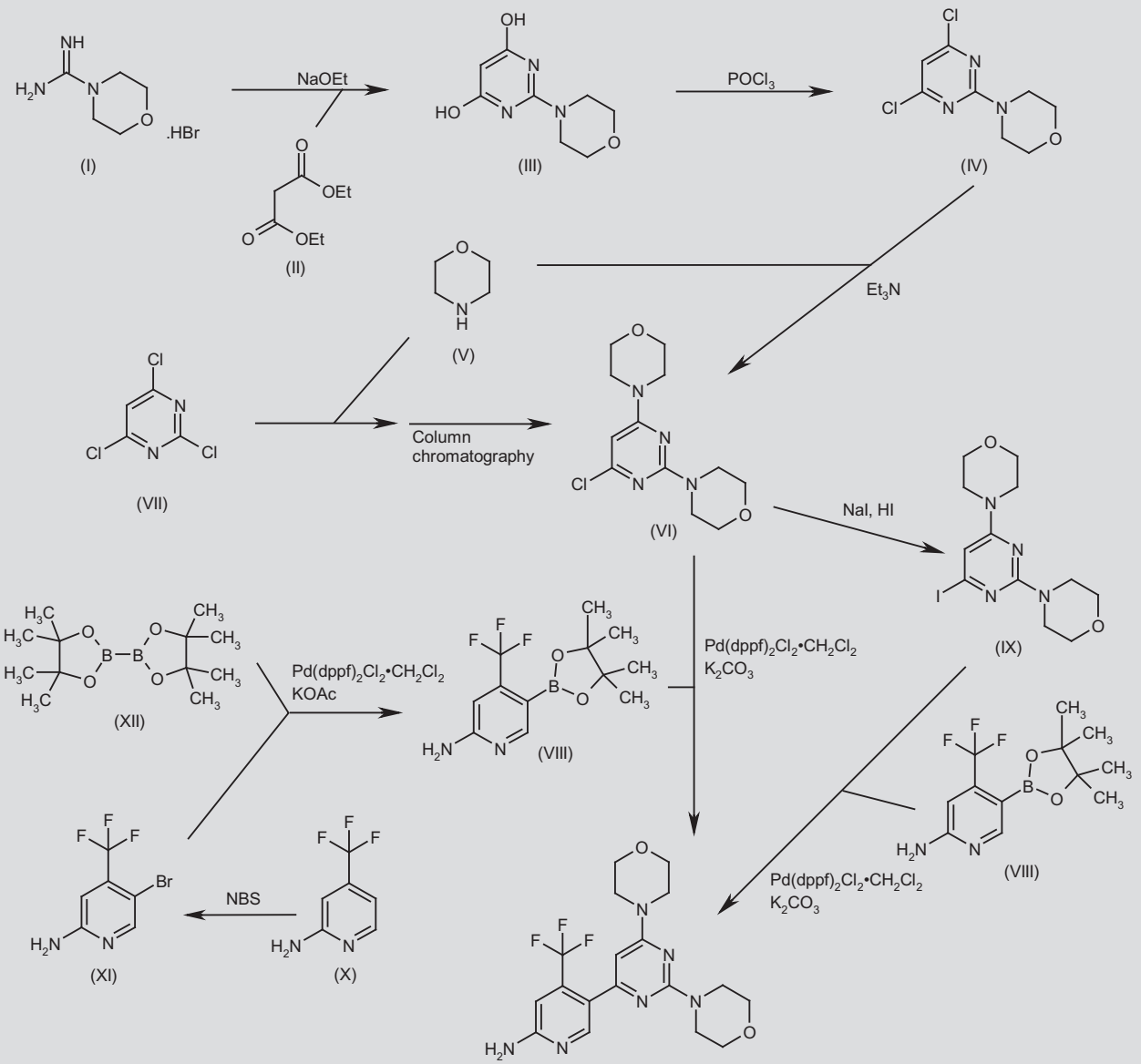
Alternatively, iodination of chloride (VI) with HI and NaI yields the corresponding iodide (IX) (3), which is also coupled with boronate (VIII) in the presence of $Pd(dppf)Cl_2·CH_2Cl_2$ and K_2CO_3 in 1,4-dioxane/H_2O at 100 °C (3). Scheme 1.

Boronate ester (VIII) is obtained by bromination of 4-(trifluoromethyl)pyridin-2-amine (X) with NBS (1, 2) in $CHCl_3$ (1, 3) or CH_2Cl_2 (2) to afford 5-bromo-4-(trifluoromethyl)pyrimidin-2-amine (XI), which is finally condensed with bis(pinacolato)diboron (XII) in the presence of $Pd(dppf)_2Cl_2·CH_2Cl_2$ and KOAc in refluxing dioxane (1, 2) or DMSO (3). Scheme 1.

Bromination of 2-amino-4-(trifluoromethyl)pyridine (X) with NBS in THF affords 2-amino-5-bromo-4-(trifluoromethyl)pyridine (XI), which

F. Azab[1], B. Muz[1], P. de la Puente[1], N. Salama[1,2] and A.K. Azab[1]. [1]Department of Radiation Oncology, Cancer Biology Division, Washington University in Saint Louis School of Medicine; [2]Division of Basic and Pharmaceutical Sciences, St. Louis College of Pharmacy, St. Louis, MO, USA, and Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, Cairo, Egypt. E-mail: aazab@radonc.wustl.edu.
*Synthesis prepared by J. Bolòs, R. Castañer. Thomson Reuters, Provença 398, 08025 Barcelona, Spain.

Scheme 1. Synthesis of Buparlisib



by *N*-acetylation with Ac_2O and DMAP in EtOAc yields the corresponding acetamide (XIII), which by sequential metalation with BuLi in THF, borylation with $(i\text{-PrO})_3B$ and hydrolysis with HCl affords the boronic acid (XIV). Suzuki cross-coupling of boronic acid (XIV) with 4-chloro-2,6-di(morpholin-4-yl)pyrimidine (VI) in the presence of $Pd(PPh_3)_4$ and K_2CO_3 in DME at 70 °C results in *N*-[4-trifluoromethyl-5-(2,6-dimorpholinopyrimidin-4-yl)pyridin-2-yl]acetamide (XV), which is finally deacetylated by treatment with HCl (4). Scheme 2.

Alternatively, Miyaura borylation of 4-chloro-2,6-di(morpholin-4-yl)pyrimidine (VI) with bis(pinacolato)diboron (XII) by means of Pd_2dba_3, KOAc and Cy_3P in acetonitrile gives boronate ester (XVI), which is finally submitted to Suzuki cross-coupling with 2-amino-5-bromo-4-(trifluoromethyl)pyridine (XI) in the presence of $Pd(dppf)Cl_2$ and Cs_2CO_3 in THF (4). Scheme 2.

BACKGROUND

The phosphatidylinositol 3-kinase (PI3K) signaling pathway is dysregulated in many tumor types, where it is associated with a poor prognosis and resistance to multiple therapies. It is also known to play a pivotal role in the initiation and progression of malignancies, enhancing cell survival by stimulating cell proliferation and inhibiting apoptosis (5, 6). The two most widely observed mechanisms of PI3K–Akt activation in cancer are activation by receptor tyrosine kinases (RTKs) and somatic mutations in specific components of the signaling pathway (7).

Upon activation by a ligand, RTKs engage and activate PI3K, which in turn converts membrane-bound phosphatidylinositol 4,5-bisphosphonate (PIP2) to phosphatidylinositol 3,4,5-triphosphonate (PIP3). PIP3 then activates Akt by phosphorylation, which Akt acts to promote cell proliferation and survival, and regulates multiple signaling pathways that maintain cell cycle, proliferation and antiapoptotic signals (5). Akt phosphorylates the forkhead box protein O (FOXO) subfamily of forkhead family transcription factors, which inhibits the transcription of proapoptotic genes, and directly inactivates proapoptotic proteins, while it activates the activity of nuclear factor NF-κB and stimulates the transcription of prosurvival genes (8). Moreover, cell cycle progression is regulated by Akt through its inhibition of the phosphorylation of the cyclin-dependent kinase inhibitors $p21^{WAF1/CIP1}$ and $p27^{KIP1}$ (9), and inhibition of glycogen synthase kinase-3 beta (GSK-3 beta) (10), which results in stimulation of cell cycle progression by stabilization of cyclin D1 (11). The most studied downstream substrate of Akt is the serine/threonine-protein kinase mTOR (mammalian target of rapamycin); Akt can directly phosphorylate and activate mTOR, as well as cause indirect activation of mTOR by phosphorylating and inactivating tuberin (TSC2) (12). TSC2 normally inhibits mTOR through the GTP-binding protein Rheb. When TSC2 is inactivated by phosphorylation, the GTP-binding protein Rheb is maintained in its GTP-bound state, allowing increased mTOR activation (13). mTOR exists in two complexes: TORC1, in which mTOR is bound to Raptor, and TORC2, in which mTOR is bound to Rictor (14). In the TORC1 complex, mTOR signals to its downstream effectors S6 kinase/ribosomal protein S6 and 4E-BP1/eIF-4E to control protein translation (15). Although mTOR is generally considered a downstream substrate of Akt, mTOR can also phosphorylate Akt when bound to Rictor in the TORC2 complex, providing a level of positive feedback on the pathway (16).

By suppressing the PI3K–Akt–mTOR pathway through its lipid phosphatase activity, phosphatase and tensin homolog (PTEN) governs a plethora of cellular processes, including survival, proliferation, energy metabolism and cellular architecture. The importance of the physiological function of PTEN is illustrated by its frequent disruption in several cancers (17).

Inhibition of PI3K is an emerging strategy in the treatment of cancer (7), and PI3K inhibitors can be divided into isoform-specific inhibitors or pan-PI3K inhibitors (18). Buparlisib (NVP-BKM-120) is a 2,6-dimorpholinopyrimidine derivative and is a potent pan-PI3K inhibitor.

PRECLINICAL PHARMACOLOGY

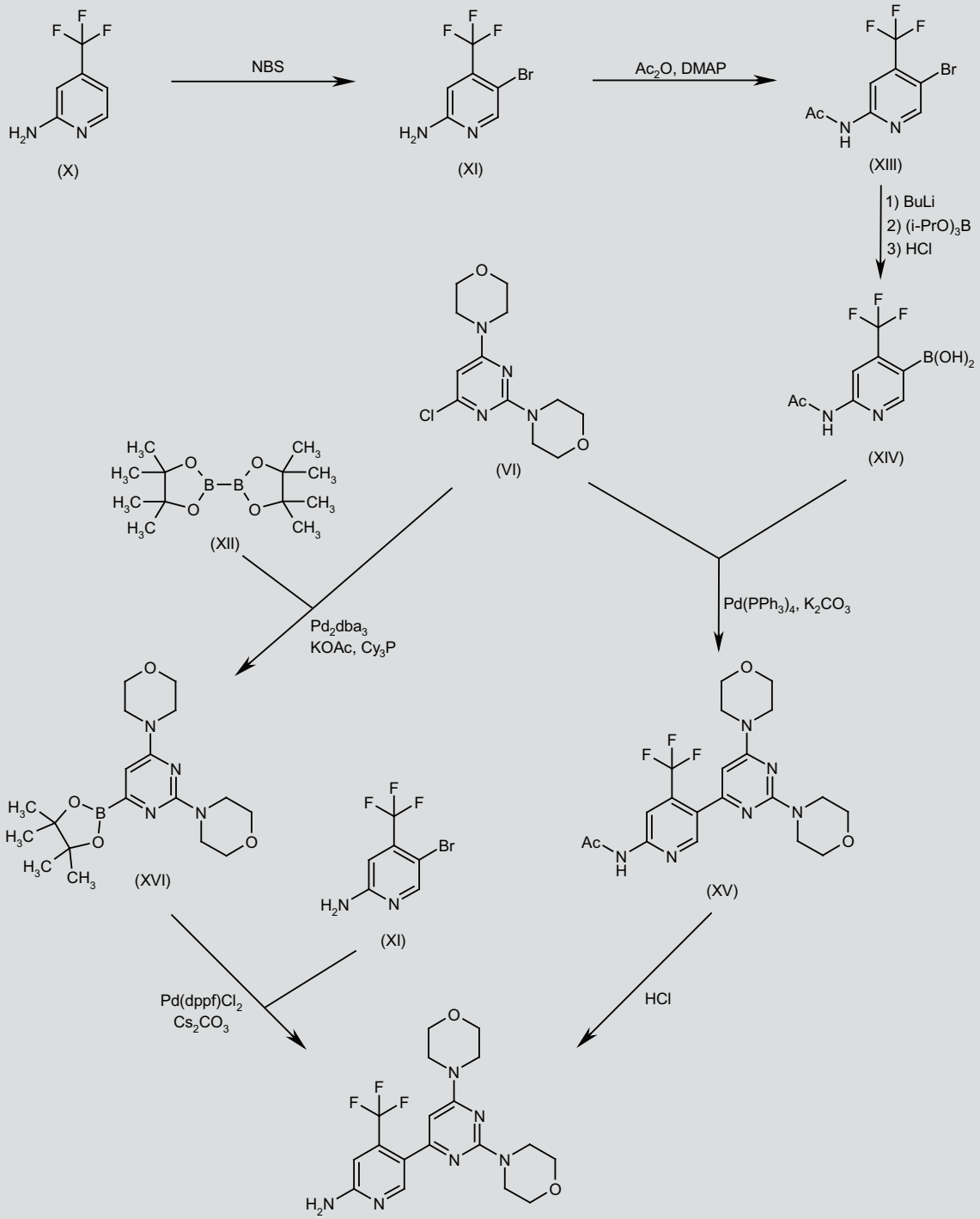
Buparlisib was shown to bind in the ATP binding site of the lipid kinase domain in the PI3K p110γ and the PI3K p110α isoforms. The binding to the α isoform suggests that there are three key hydrogen bond interactions formed by the oxygen of the 2-morpholino group and by the exocyclic nitrogen in the 6-position pyridyl substituent. The morpholine oxygen functions as a hydrogen bond acceptor with the backbone amino group of Val851 in the hinge domain of the PI3K p110α isoform. A similar interaction is observed in all the published structures with ATP and with known inhibitors. The 6-pyridyl exocyclic nitrogen (as a donor) binds to Asp810 and Asp933 in the catalytic region. A fourth interaction could be modeled, as high-resolution structures of p110γ are frequently observed to contain a water molecule forming a hydrogen bond bridge between Tyr836 and Asp810. Consistent with its mechanism of action, buparlisib decreases the cellular levels of p-Akt in mechanistic models and relevant tumor cell lines, as well as downstream effectors in a concentration-dependent and pathway-specific manner (19).

Buparlisib is approximately equipotent against the class IA PI3Ks alpha, beta and delta and modestly less potent against the class IB gamma isoform. The compound also shows comparable potency against activating p110α somatic mutations that have been described in a wide array of human cancers. Buparlisib is significantly less potent in biochemical assays against the PI3K class III family member hVps34, the related class IV protein kinases mTOR, serine/threonine-protein kinase ATR and DNA-dependent protein kinase (DNA-PK), and the distinct lipid kinase phosphatidyl 4-kinase beta (PI4Kbeta). Buparlisib was shown to be mostly inactive against all the kinases tested in an in-house selectivity panel, with the exception of macrophage colony-stimulating factor 1 receptor (CSF-1R). Buparlisib was further profiled in the Ambit kinase competition panel, in which ephrin-type A receptor 2 (EPHA2) and fibroblast growth factor receptor 2 (FGFR-2) kinases were found to be inhibited by more than 90% at a concentration of 1 μmol/L. Such as for CSF-1R, these hits were not reconfirmed in FGFR-2 and EPHA2 cellular autophosphorylation assays. Therefore, buparlisib can be considered as a selective pan-class I PI3K inhibitor (19).

Buparlisib downregulates PI3K downstream signaling by inhibiting the phosphorylation of Akt and p-S6R, which resulted in reduced tumor proliferation in breast cancer models (20-23). Despite suppression of Akt phosphorylation as a resistance mechanism, cancer cells exhibited activation of the mitogen-activated protein kinase (MAPK) pathway in dyslipidemia-induced tumor growth and metastasis in murine mammary Mvt-1 cells (24), lung cancer (25), c-Myc-driven medulloblastoma (26), glioma (27) and *PTEN*-null human glioblastoma U-87 MG cells (19). However, in gastric cancer cells harboring *KRAS* mutations, buparlisib treatment increased p-Akt by subsequent abrogation of feedback inhibition by stabilizing insulin receptor substrate, and increased activation of p-ERK and p-STAT3 was observed (28).

Buparlisib induced apoptosis in a concentration-dependent manner in breast cancer (20), glioma (27), gastrointestinal cancer (29) and

Scheme 2. Synthesis of Buparlisib



multiple myeloma (30). Buparlisib induced apoptosis through activation of caspase, followed by downregulation of the antiapoptotic protein E3 ubiquitin-protein ligase XIAP and upregulation of the proapoptotic protein Bcl-2-like protein 11 (BIM) (30). Buparlisib restricted the growth of highly and moderately proliferative cell lines in a concentration-dependent manner (31). It induced a G_2 shift in gastrointestinal cancer cells (29) and changed the expression of mitotic genes to induce G_2-M arrest in melanoma cells (32). In multiple myeloma, buparlisib induced G_1 cell cycle arrest through upregulation of p27 and downregulation of cyclin D1 (30).

In murine breast cancer models, buparlisib inhibited tumor growth via inhibition of PI3K–Akt–mTOR signaling in the hyperinsulinemic MKR mouse model of breast cancer (21), led to a significant reduction in tumor vasculature leakiness from the tumor tissue (19) and controlled metastatic growth in multiple organs, resulting in a significant proportion of *Rag2*$^{-/-}$;*Il2rg*$^{-/-}$ mice free from brain and bone metastases for its penetration of the blood–brain barrier (33). In a murine lung cancer model, buparlisib alleviated dyslipidemia-induced tumor growth and metastasis in the Mvt-1 model, with a concomitant decrease in PI3K–Akt signaling (24), and it synergized with everolimus in a mouse xenograft model (34). In a murine glioma model, buparlisib increased median survival from 26 days (control cohort) to 38 and 48 days (treated cohorts) (27). In a murine multiple myeloma model, treatment with buparlisib significantly inhibited tumor growth and prolonged the survival of myeloma-bearing mice (30). In gastric cancer models, buparlisib led to tumor regressions, with no increase in the mitotic index (32). And in a mouse soft tissue sarcoma model, buparlisib elicited a partial response in 50% of tumors and produced a robust delay in tumor growth kinetics (35).

Buparlisib synergized with mTOR inhibitors in lung cancer (25, 34) and melanoma (36), with dexamethasone in multiple myeloma (30), with STAT3 inhibitors in *KRAS*-mutant gastric cancer (28), with PARP inhibitors in breast cancer (22, 23), with MAPK inhibitors in breast cancer (19, 20, 23), with HER2 inhibitors in breast cancer (19, 37), with cytotoxic agents such as docetaxel and temozolomide in breast cancer (19), and with SMO inhibitors in medulloblastoma (38).

Buparlisib at high concentrations induces cell death in various cellular systems, irrespective of their level of PI3K addiction. At 5- to 10-fold, tubulin polymerization assays and nuclear magnetic resonance binding studies revealed that the agent inhibited microtubule dynamics upon direct binding to tubulin, which resulted in G_2 cell cycle arrest. In in vitro settings, the consequences of the off-target activity started to manifest at concentrations above 1 μM and at doses above 50 mg/kg in mouse models. However, in vivo models showed that daily treatment of mice with doses of up to 40 mg/kg led to tumor regressions with no increase in the mitotic index. Thus, strong antitumor activity can be achieved in PI3K-dependent models at exposures that are below those necessary to engage the off-target activity (32).

PHARMACOKINETICS AND METABOLISM

Buparlisib is rapidly absorbed after oral administration, with the median time to reach peak plasma concentrations (t_{max}) between 0.5 and 4 hours postdose. T_{max} appeared to be independent of dose and did not change after multiple oral doses. Physiologically based models predicted that a fraction of approximately 0.95 of the dose was absorbed, with an absolute oral bioavailability of more than 90%.

Buparlisib exposure within a dosing interval (AUC_{0-24}) and C_{max} was similar between days 8 and 28 of daily oral dosing, indicating the absence of significant drug accumulation after day 8. An approximately dose-proportional increase in C_{max} and AUC_{0-24} was observed across the entire dose range. Interpatient variability (CV%) in C_{max} and AUC_{0-24} differed at each dose level but was moderate and generally approximately 40%.

After reaching the peak drug concentration (C_{max}), buparlisib plasma concentrations decreased in a biexponential manner with an apparent long terminal elimination half-life ($t_{1/2}$). The agent accumulated threefold in achieving steady state, consistent with a half-life of ~40 hours. Apparent total body clearance from plasma at steady state (calculated as dose/AUC_{0-24}) was low (~5.0 L/h). Physiologically based models predicted a hepatic clearance (CL_{hep}) of approximately 2 to 8 L/h, respectively, with an absolute oral bioavailability of > 90%. These predictions are in close agreement with the clearance of 5 L/h and the first-pass hepatic extraction of < 10%.

SAFETY

In a phase I dose-escalation trial of buparlisib in 35 patients with advanced solid tumors, the maximum tolerated dose (MTD) was 100 mg/day. Grade 3/4 adverse events (AEs), regardless of causality, were observed in 22 patients (63%). Of the grade 3/4 AEs, regardless of causality, rash (11%), hyperglycemia (9%), performance status decrease (9%), mood alteration (9%; including mood altered [6%] and affective disorder [3%]) and pruritus (6%) were observed in two or more patients. The majority of treatment-related AEs were observed at dose levels ≥ 100 mg. Five patients (14%) experienced serious AEs considered to be treatment-related: hyperglycemia (80 mg, 150 mg [n = 2]), skin rash (150 mg) and diarrhea (100 mg). All four deaths on study resulted from disease progression (39).

In preclinical settings, at high doses, buparlisib has off-target activity for inhibiting tubulin polymerization and consequently inhibition of mitosis and G_2/M arrest (32). In the clinical setting, at the MTD of 100 mg/day, the plasma exposure (AUC: 56 hμmol/L) (39) lies below the exposure necessary to transiently engage the off-target in a mouse model (AUC > 65 hμmol/L) (28). These findings suggest that, in patients, the threshold for microtubule-destabilizing activity of this compound is not reached. Therefore, it is anticipated that efficacy in patients will solely stem from PI3K inhibition.

CLINICAL STUDIES

In a phase I clinical trial of buparlisib in 35 patients with advanced solid tumors, patients had histologically confirmed advanced tumors failing standard therapy, one or more lesions as defined by RECIST, age ≥ 18 years, life expectancy ≥ 12 weeks, World Health Organization performance status ≤ 2, adequate bone marrow, hepatic and renal function, and fasting plasma glucose levels ≤ 140 mg/dL (7.8 mmol/L). A representative tissue specimen for analysis of tumor molecular status was required. Key exclusion criteria were corticosteroid treatment ≤ 2 weeks before starting buparlisib, clinically manifest diabetes, including a history of gestational diabetes,

and prior treatment with a PI3K inhibitor. Thirty-one of 35 patients were evaluable for response; baseline and post-baseline target lesion radiological assessments were available for 24 patients by central review. One patient with triple-negative breast cancer and a *KRAS* mutation achieved a partial response on a dose of 100 mg/day. Sixteen patients (of 31; 52%) had stable disease for more than 6 weeks, including 5 with colorectal cancer and 5 with breast cancer. Seven patients had been on the study for ≥ 8 months, most at 100 mg/day (two patients each with breast cancer and colorectal cancer and one patient each with prostate cancer, angiosarcoma and lung adenocarcinoma). Five of these patients had tumors with PI3K pathway dependence (PTEN low/no protein expression or *PIK3CA* gene mutation). This study demonstrated feasibility and proof-of-concept for class I PI3K inhibition in patients with advanced cancers.

Buparlisib has also been tested in a phase II clinical trial used as therapy for patients with recurrent/metastatic squamous cell carcinoma of the head and neck (SCCHN). This is the first PI3K inhibitor tested in clinical trials for head and neck cancer, and it was a multicenter, single-arm phase II study to determine the antitumor effects of buparlisib in patients with recurrent and/or metastatic SCCHN who failed prior platinum-based chemotherapy regimens.

A second phase II clinical trial is testing buparlisib as a single agent in first-line therapy in advanced, metastatic or recurrent endometrial cancer**.** Alterations of the PI3K–PTEN–Akt pathway have been identified in many cancers, including endometrial and ovarian cancers. Tumors with PI3K mutations have demonstrated sensitivity to this compound, therefore justifying the use of this agent in subjects with endometrial or ovarian, fallopian tube or primary peritoneal cancer. This study is not yet open for participant recruitment.

SOURCE

Novartis (CH).

DISCLOSURES

The authors state no conflicts of interest.

REFERENCES

1. Pick, T., Barsanti, P., Iwanowicz, E. et al. (Novartis AG). *Pyrimidine derivatives used as PI-3 kinase inhibitors.* EP 1984350, EP 2261223, JP 200952764, US 2010249126, US 8217035, US 2012225859, WO 2007084786.
2. Burger, M.T., Pecchi, S., Wagman, A. et al. *Discovery of BKM120, a pan class I PI3 kinase inhibitor in phase I/II clinical trials*. 240th ACS Natl Meet (Aug 22-26, Boston) 2010, Abst MEDI 489.
3. Vu, A.T., Morris, J. Malhotra, S.V. *Efficient and improved synthesis of a PI3K inhibitor anticancer agent.* 241st ACS Natl Meet (March 27-31, Anaheim) 2011, Abst ORGN 115.
4. Calienni, J.V., De La Cruz, M., Flubacher, D. et al. (Novartis AG). *Manufacturing process for pyrimidine derivatives*. WO 2012044727.
5. LoPiccolo, J., Blumenthal, G.M., Bernstein, W.B., Dennis, P.A. *Targeting the PI3K/Akt/mTOR pathway: Effective combinations and clinical considerations.* Drug Resist Updat 2008, 11(1-2): 32-50.
6. Feldman, M.E., Shokat, K.M. *New inhibitors of the PI3K-Akt-mTOR pathway: Insights into mTOR signaling from a new generation of Tor kinase domain inhibitors (TORKinibs).* Curr Top Microbiol Immunol 2010, 347: 241-62.
7. Engelman, J.A. *Targeting PI3K signalling in cancer: Opportunities, challenges and limitations.* Nat Rev Cancer 2009, 9(8): 550-62.
8. Korbecki, J., Baranowska-Bosiacka, I., Gutowska, I., Chlubek, D. *Biochemical and medical importance of vanadium compounds.* Acta Biochim Pol 2012, 59(2): 195-200.
9. Izutani, Y., Yogosawa, S., Sowa, Y., Sakai, T. *Brassinin induces G1 phase arrest through increase of p21 and p27 by inhibition of the phosphatidylinositol 3-kinase signaling pathway in human colon cancer cells.* Int J Oncol 2012, 40(3): 816-24.
10. Kim, L., Kimmel, A.R. *GSK3, a master switch regulating cell-fate specification and tumorigenesis.* Curr Opin Genet Dev 2000, 10(5): 508-14.
11. Liang, J., Slingerland, J.M. *Multiple roles of the PI3K/PKB (Akt) pathway in cell cycle progression.* Cell Cycle 2003, 2(4): 339-45.
12. Gomez-Pinillos, A., Ferrari, A.C. *mTOR signaling pathway and mTOR inhibitors in cancer therapy.* Hematol Oncol Clin North Am 2012, 26(3): 483-505.
13. Orlova, K.A., Crino, P.B. *The tuberous sclerosis complex.* Ann N Y Acad Sci 2010, 1184: 87-105.
14. Proud, C.G. *mTOR signalling in health and disease.* Biochem Soc Trans 2011, 39(2): 431-6.
15. Julien, L.A., Carriere, A., Moreau, J., Roux, P.P. *mTORC1-activated S6K1 phosphorylates Rictor on threonine 1135 and regulates mTORC2 signaling.* Mol Cell Biol 2010, 30(4): 908-21.
16. Cybulski, N., Hall, M.N. *TOR complex 2: A signaling pathway of its own.* Trends Biochem Sci 2009, 34(12): 620-7.
17. Song, M.S., Salmena, L., Pandolfi, P.P. *The functions and regulation of the PTEN tumour suppressor.* Nat Rev Mol Cell Biol 2012, 13(5): 283-96.
18. Kurtz, J.E., Ray-Coquard, I. *PI3 kinase inhibitors in the clinic: An update.* Anticancer Res 2012, 32(7): 2463-70.
19. Maira, S.M., Pecchi, S., Huang, A. et al. *Identification and characterization of NVP-BKM120, an orally available pan-class I PI3-kinase inhibitor.* Mol Cancer Ther 2012, 11(2): 317-28.
20. Sanchez, C.G., Ma, C.X., Crowder, R.J. et al. *Preclinical modeling of combined phosphatidylinositol-3-kinase inhibition with endocrine therapy for estrogen receptor-positive breast cancer.* Breast Cancer Res 2011, 13(2): R21.
21. Gallagher, E.J., Fierz, Y., Vijayakumar, A., Haddad, N., Yakar, S., LeRoith, D. *Inhibiting PI3K reduces mammary tumor growth and induces hyperglycemia in a mouse model of insulin resistance and hyperinsulinemia.* Oncogene 2012, 31(27): 3213-22.
22. Juvekar, A., Burga, L.N., Hu, H. et al. *Combining a PI3K inhibitor with a PARP inhibitor provides an effective therapy for, BRCA1-related breast cancer.* Cancer Discov 2012, 2(11): 1048-63.
23. Ibrahim, Y.H., García-García, C., Serra, V. et al. *PI3K inhibition impairs BRCA1/2 expression and sensitizes BRCA-proficient triple-negative breast cancer to PARP inhibition.* Cancer Discov 2012, 2(11): 1036-47.
24. Alikhani, N., Ferguson, R.D., Novosyadlyy, R., Gallagher, E.J., Scheinman, E.J., Yakar, S., Leroith, D. *Mammary tumor growth and pulmonary metastasis are enhanced in a hyperlipidemic mouse model.* Oncogene 2012.
25. Zito, C.R., Jilaveanu, L.B., Anagnostou, V. et al. *Multi-level targeting of the phosphatidylinositol-3-kinase pathway in non-small cell lung cancer cells.* PLoS One 2012, 7(2): e31331.
26. Pei, Y., Moore, C.E., Wang, J. et al. *An animal model of MYC-driven medulloblastoma.* Cancer Cell 2012, 21(2): 155-67.

27. Koul, D., Fu, J., Shen, R. et al. *Antitumor activity of NVP-BKM120—A selective pan class I PI3 kinase inhibitor showed differential forms of cell death based on p53 status of glioma cells.* Clin Cancer Res 2012, 18(1): 184-95.

28. Park, E., Park, J., Han, S.W., Im, S.A., Kim, T.Y., Oh, D.Y., Bang, Y.J. *NVP-BKM120, a novel PI3K inhibitor, shows synergism with a STAT3 inhibitor in human gastric cancer cells harboring KRAS mutations.* Int J Oncol 2012, 40(4): 1259-66.

29. Mueller, A., Bachmann, E., Linnig, M., Khillimberger, K., Schimanski, C.C., Galle, P.R., Moehler, M. *Selective PI3K inhibition by BKM120 and BEZ235 alone or in combination with chemotherapy in wild-type and mutated human gastrointestinal cancer cell lines.* Cancer Chemother Pharmacol 2012, 69(6): 1601-15.

30. Zheng, Y., Yang, J., Qian, J. et al. *Novel phosphatidylinositol 3-kinase inhibitor NVP-BKM120 induces apoptosis in myeloma cells and shows synergistic anti-myeloma activity with dexamethasone.* J Mol Med (Berl) 2012, 90(6): 695-706.

31. Kelly, C.J., Hussien, K., Muschel, R.J. *3D tumour spheroids as a model to assess the suitability of [18F]FDG-PET as an early indicator of response to PI3K inhibition.* Nucl Med Biol 2012, 39(7): 986-92.

32. Brachmann, S.M., Kleylein-Sohn, J., Gaulis, S. et al. *Characterization of the mechanism of action of the pan class I PI3K inhibitor NVP-BKM120 across a broad range of concentrations.* Mol Cancer Ther 2012, 11(8): 1747-57.

33. Nanni, P., Nicoletti, G., Palladini, A. et al. *Multiorgan metastasis of human HER-2(+) breast cancer in Rag2(-)/(-);Il2rg(-)/(-) mice and treatment with PI3K inhibitor.* PLoS One 2012, 7(6): e39626.

34. Ren, H., Chen, M., Yue, P. et al. *The combination of RAD001 and NVP-BKM120 synergistically inhibits the growth of lung cancer in vitro and in vivo.* Cancer Lett 2012, 325(2): 139-46.

35. Kim, S., Dodd, R.D., Mito, J.K., Ma, Y., Kim, Y., Riedel, R.F., Kirsch, D.G. *Efficacy of phosphatidylinositol-3 kinase inhibitors in a primary mouse model of undifferentiated pleomorphic sarcoma.* Sarcoma 2012, 2012: 680708.

36. Aziz, S.A., Jilaveanu, L.B., Zito, C., Camp, R.L., Rimm, D.L., Conrad, P., Kluger, H.M. *Vertical targeting of the phosphatidylinositol-3 kinase pathway as a strategy for treating melanoma.* Clin Cancer Res 2010, 16(24): 6029-39.

37. Miller, T.W., Balko, J.M., Fox, E.M. et al. *ERalpha-dependent E2F transcription can mediate resistance to estrogen deprivation in human breast cancer.* Cancer Discov 2011, 1(4): 338-51.

38. Buonamici, S., Williams, J., Morrissey, M. et al. *Interfering with resistance to smoothened antagonists by inhibition of the PI3K pathway in medulloblastoma.* Sci Transl Med 2010, 2(51): 51ra70.

39. Bendell, J.C., Rodon, J., Burris, H.A. et al. *Phase I, dose-escalation study of BKM120, an oral pan-class I PI3K inhibitor, in patients with advanced solid tumors.* J Clin Oncol 2012, 30(3): 282-90.