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A First Time in Human Trial of GSK2636771, a PI3Kβ selective Inhibitor, in patients with advanced solid tumors

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JM declares no conflict of interest; GG is an employee of GSK and holds stocks and shares in GSK

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ABSTRACT (*150 words maximum; current: 174*)

Purpose: This clinical trial evaluated the safety, pharmacokinetic and pharmacodynamic effects and potential anti-tumor activity of the PI3Kβ inhibitor GSK2636771 to define the recommended phase II dose (RP2D).

Patients and Methods*:* Patients with phosphatase and tensin homolog (PTEN)-deficient advanced solid tumors received escalating doses of GSK2636771 (25–500 mg QD), followed by expansion cohorts at the RP2D. Retrospective sequencing of tumor samples was performed to define the optimal target population.

Results*:* 65 patients were enrolled; dose-limiting toxicities were hypophosphatemia and hypocalcemia. Common adverse events included diarrhea (48%), nausea (40%) and vomiting (31%). 400 mg QD was identified as the RP2D. Phospho/total AKT ratio decreased in tumor biopsies and platelet rich plasma upon treatment with GSK2636771. A radiological partial response (as per RECIST 1.1) was observed in a patient with CRPC and *PIK3CB* amplification, and prolonged stable disease or disease control was noted in several others.

Conclusion*:* GSK2636771 administered orally at 400 mg QD resulted in sufficient exposure and target inhibition with a manageable safety profile. Genomic aberrations of *PIK3CB* may predict response to monotherapy.

Statement of significance (50 words maximum; currently 47)

First-time-in-human pharmacokinetic and safety data indicate that the PI3Kβ-selective inhibitor, GSK2636771, may provide a viable therapeutic option for patients with phosphatase and tensin homolog-deficient advanced solid tumors and/or *PIK3CB* genomic aberrations. Further clinical studies of GSK2636771 in combination with other agents in different cancers are currently underway.

INTRODUCTION

Activation of phosphoinositide 3 kinase (PI3K)/Akt/mTOR signaling [1], most commonly by activating mutations of *PI3K/AKT* family members or loss of the phosphatase and tensin homolog (PTEN) phosphatase function, contributes to the progression of many cancers [2-5]. Inhibition of PI3K signaling has been challenging therapeutically, and inhibitors of PI3K have had limited clinical success [6,7]. Reasons for this include biological feedback loops permitting the tumor to reactivate the pathway [8,9], activation of alternative pathways [10], and the non-specificity of pan-PI3K tyrosine kinase inhibitors, resulting in a plethora of off-target toxicities, which limit administration of active doses in a continuous manner [11]. PI3K is composed of a heterodimer between a p110 catalytic subunit and a p85 regulatory subunit. The four described isoforms of the catalytic subunit are p110α, p110β, p110γ and p110δ, encoded by genes *PIK3CA*, *PIK3CB*, *PIK3CG* and *PIK3CD* respectively [12]. PI3K-isoform-selective inhibitors have been developed in attempts to reduce off-target toxicity [13].

Loss of PTEN function has been observed in a number of cancers, including glioblastoma, prostate, endometrial, melanoma and breast cancers. Preclinical studies have indicated that the PI3Kβ isoform (containing the p110β catalytic subunit) is the critical lipid kinase that drives PI3K pathway activation, cell growth and survival in PTEN-deficient tumor cells [14-17]. Highly selective PI3Kβ inhibition is therefore hypothesized to have utility against PTEN-deficient cancers, whilst avoiding toxicities associated with inhibition of other PI3K isoforms [18] or other off-target effects. This is likely to minimize toxicities and maximize therapeutic efficacy by enabling administration of appropriate doses and rational drug combinations with other agents, such as androgen receptor (AR) antagonists in PTEN deficient prostate cancer [19-21], or erbB2 inhibitors and hormonal treatments in breast cancer [22-25].

GSK2636771 (Figure 1A), is a potent, orally bioavailable, adenosine triphosphate (ATP) competitive, selective inhibitor of PI3Kβ with an apparent Ki value of 0.89 nM,

>900-fold selectivity over p110α and p110γ, and >10-fold selectivity over p110δ isoforms, while sparing other PI3K superfamily kinases (Figure 1B).

Here we present preclinical data characterizing the selectivity of GSK2636771 in cell cultures and murine xenograft models, together with the results of a dose-finding, first-in-human study of GSK2636771 in patients with PTEN-deficient or *PIK3CB* genomically altered advanced solid tumors. The aim of the first-in-human study was to further characterize the tolerability, safety and pharmacokinetic-pharmacodynamic (PK-PD) profile of GSK2636771, while also assessing its antitumor activity. We also pursued genomics analyses to assess any alterations as putative predictive biomarkers of antitumor response.

RESULTS

Preclinical Studies

GSK2636771 selectively inhibited the growth of PTEN-deficient cancer cells in a cell line panel spanning multiple histologies (Figure 1C), and inhibition of protein kinase B (AKT) and ribosomal S6 kinase phosphorylation was observed in a concentration- and time-dependent manner in PTEN-deficient cells (Figures 1D and 1E). GSK2636771 had no effect on mitogen-activated protein kinase (MAPK) signaling, as evidenced by measurement of extracellular signal-regulated kinase (ERK) phosphorylation. When administered orally in mice bearing PC-3 prostate tumor xenografts, GSK2636771 resulted in stable disease and/or tumor growth inhibition, and a dose- and time-dependent PK-PD response was observed (Figures 1F and 1G). Importantly, GSK2636771 did not elevate glucose or insulin levels in mice compared with the pan PI3K/mammalian target of rapamycin (mTOR) inhibitor, GSK2126458 (Supplementary Figure 1).

First-time-in-human study

Patients and administered treatments

Overall, 65 patients were enrolled and received at least one dose of study medication: three patients in Part 1 (dose selection), 50 in Part 2 (dose-escalation and additional PD exploratory cohorts) and 12 as part of the expansion cohorts in Part 3. Baseline patient demographics and characteristics are summarized in **Table 1**. Briefly, the median age of the study population was 62 years (range 30–79), 26/65 (40%) patients were female, and the most common tumor types were colorectal (n=23, 35%) and prostate cancers (n=12, 18%). All (100%) patients had received at least one previous anti-cancer treatment, 36 (57%) had received >4 anti-cancer treatments, and all (100%) patients had undergone a surgical procedure. In total, seven dose levels (25–500 mg once daily [QD]) were investigated. Median time on treatment was 55 days (range 5 to 478).

Selection of the starting dose for dose-escalation stage

Three patients were enrolled in Part 1 of the study and received a single dose of 25 mg of GSK2636771. The geometric mean area under the concentration-time curve from zero (pre-dose) to 24 hours ($AUC_{[0–24]}$) was 15.7 µg*hr/mL, which was within the pre-specified target range of 10–50 µg*hr/mL. Consequently, 25 mg QD was selected as the initial dose for the dose-escalation stage.

Safety, Tolerability and Dose Limiting Toxicities

No Dose Limiting Toxicities (DLTs) were observed in any patient receiving 25–350 mg QD of GSK2636771. Dose-escalation then continued to 500 mg QD, where 3 of 4 treated patients experienced a DLT (hypocalcemia [Grades 2 and 3], and hypophosphatemia [Grade 3]), during the second to third week of continuous treatment. One patient also experienced a Grade 1 creatinine elevation. These toxicities, indicative of renal tubular damage, resolved after GSK2636771

discontinuation (except for one with normalized phosphate levels but persisting Grade 1 hypocalcemia) and two of the three patients were able to continue GSK2636771 treatment at a lower dose. An intermediate lower dose of 400 mg QD was explored (n=6), and no DLTs were observed. As such, 400 mg QD was selected as the RP2D.

All 65 patients experienced at least one AE during the study; the most common AEs across all dose levels were gastrointestinal (diarrhea [n=31, 48%], nausea [n=26, 40%], vomiting [n=20, 31%], and fatigue (n=16 [25%]). Overall, ten (15%) patients had treatment permanently discontinued due to an adverse event, most commonly fatigue (n=3, 5%; all other n=1 [2%]). Forty-two serious AEs (SAEs) occurred in 24 (37%) patients. Nine SAEs were considered related to the study drug (nausea, vomiting, fatigue, increased creatinine, decreased appetite, hypocalcemia, hypophosphatemia, urinary retention and pruritic rash; all n=1; all Grade 2 or 3 except for increased creatinine [Grade 1]), and there was one (2%) fatal SAE (dyspnea) that was not considered related to the study drug. Eight (12%) additional deaths occurred during the study, all of which were considered related to the underlying disease.

Hyperglycemia, which has been reported when targeting other nodes in the PI3K/AKT/mTOR pathway,[29] was reported in 36 (55%) patients receiving GSK2636771 treatment and was predominantly mild in severity; only two (3%) hyperglycemia events were recorded as AEs. Cutaneous toxicity was uncommon: 10 cases (15%) of skin rash were documented across all dose levels (four of which were in the GSK2636771 400 mg QD group). Rashes were primarily maculopapular and caused pruritus. Other than the aforementioned cases of hypophosphatemia and hypocalcemia, evidence for renal tubular toxicity also included proteinuria, which was reported in four (4/65, 6%) patients: one in each of the GSK2636771 50 mg, 200 mg, 350 mg, and 500 mg QD dose groups.

Pharmacokinetics and pharmacodynamics (PK-PD)

Following a single run-in dose of GSK2636771, drug exposure (C_{max}, AUC) increased dose proportionally up to 350 mg, with below-proportional increments above this dose. The median T_{max} was 4 hours (range 1–10 hours). Blood concentrations declined in a monophasic manner with a geometric mean $t_{½}$ between 13–23 hours. Similar dose-proportional findings (for both $AUC_{(0–τ)}$ and C_{max}) were observed after repeated daily oral dosing at Day 22, with PK parameters suggesting steady-state had been achieved (**Table 3**). PK parameters for the PD cohorts were similar to those observed in the dose-escalation cohorts.

GSK2636771 doses above 200 mg consistently resulted in blood concentrations greater than 0.6 µg/mL, the level predicted to robustly inhibit PI3Kβ. At the RP2D of 400 mg QD, pre-dose concentrations remained above 3.04 µg/mL from Week 2 onward, T_{max} ranged between 1.02 and 5.8 hours post-dose, and $AUC_{(0–τ)}$ had a geometric mean of 205 µg/mL.

Inhibition of PI3K signaling was observed in platelet rich plasma (PRP) with GSK2636771 doses of 100–500 mg QD; the median percentage decrease from baseline at all post-dose time points was ≥61% for pSer473/Total AKT (**Figure 2A**) and ≥60% for pSer9/Total GSK3β (**Figure 2B**). At Day 1, the inhibitory effects were shown to be greatest 1–2 hours post-dose with inhibition duration increasing from 10 hours to 24 hours at GSK2636771 doses ≥100 mg **(Supplementary Table 1).**

Decreases in pSer473 AKT and its downstream target (pThr246 PRAS40) were observed in paired tumor biopsies from 4/5 (80%) patients who received the RP2D of 400 mg QD (**Figures 2C and 2D**). Decreases in pSer235/236 S6RP and pThr308 AKT were also observed in 2 of these 5 (40%) patients (**Figures 2C and 2D**).

Antitumor activity

The investigator-assessed best responses (based on Response Evaluation Criteria in Solid Tumors [RECIST] 1.1 criteria) showed progressive disease in 35 (54%) patients and stable disease in 21 (32%) patients. No complete responses were reported. One patient with metastatic-castration resistant prostate cancer treated with GSK2636771 200 mg QD during the dose-escalation phase experienced a partial response, as well as a 78% fall in his prostate-specific antigen levels. The response was durable with progression after 16 months of treatment (**Figure 3A–C**). In addition, 9 (14%) patients treated with GSK2636771 remained on therapy and free of progression for at least 6 months, including 2 prostate cancer patients who received treatment for >1 year.

Association between antitumor activity and genomic biomarkers

Archival, diagnostic, tumor biopsy samples from 55 patients participating in the study were retrieved. Of those, 48 (87%) passed quality control for next-generation sequencing. Overall, five (10%) patients had a *PIK3CB* copy number gain or a mutation predicted to activate *PIK3CB* (**Figure 3D)**.

Overall, 5/7 (57%) patients with *PIK3CB* genomic aberrations were on trial for ≥6 months. Three of these patients had CRPC, and remained on GSK2636771 treatment for 34, 57 and 68 weeks (the latter being the single patient recording a radiological partial response). One patient with cervical cancer received 24 weeks of GSK2636771 treatment, and showed a differential radiological response in the lymph nodes (best overall response: stable disease).

The p.L1049R mutation identified in a patient with castration-resistant prostate cancer (CRPC) is homologous to the *PIK3CA* activating mutation p.H1047R (**Figure 3E and 3F**) and has been previously observed in a glioblastoma patient from the Cancer Genome Atlas.[30] In order to assess the functional relevance of the p.L1049R *PIK3CB* mutation, we transduced PC3 cells with pHTBBV1.1 (using baculovirus gene transfer

into mammalian cells) expressing wildtype *PI3Kβ* or the p.L1049R mutant at a range of multiplicity of infections. After 12 hours, higher levels of pAKT were observed in cells with the p.L1049R mutation compared with wild-type cells (**Figure 3G**), suggesting an activating and potentially driving function for this mutation. Similar findings have now been reported for other mutations in the same region of *PIK3CB* [31].

Lastly, we analyzed the frequency of genomic events in other tumor suppressor and cancer promoting genes in the trial population. *PIK3KCA* activating mutations were identified in 11/48 (23%) patients, with one additional patient showing a *PI3KCA* amplification; nine of these tumors harbored mutations in the RAS/RAF pathway and three in ataxia telangiectasia mutated (ATM), a key element of DNA damage response (**Figure 3D**). Interestingly, all these were mutually exclusive with *PIK3CB* aberrations in this population and did not correlate with anti-tumor responses.

DISCUSSION

In this first-time-in-human trial of GSK2636771, we identified DLTs and established the RP2D for this orally available, selective, PI3Kβ inhibitor. Renal tubular damage, presenting in the form of hypophosphatemia, hypocalcemia and proteinuria, was dose dependent, reversible and manageable. This is in contrast with toxicities typically reported for PI3K inhibitors. Furthermore, no hemorrhagic events or coagulation alterations were observed, despite preclinical data indicating that PI3Kβ plays an important role in adenosine diphosphate-induced platelet aggregation.[32]

Sufficient systemic exposure to, and target inhibition by, GSK2636771 were demonstrated at tolerated doses. Repeat-dose exposure appeared to increase in a dose-proportional manner. GSK2636771 doses >200 mg QD consistently resulted in blood concentrations above those expected to robustly inhibit PI3Kβ. The observed inhibitory effect of GSK2636771 on pAKT (Ser473) and other biochemical markers (e.g. pGSK3β [Ser9], pPRAS40) in PRP confirmed an effective modulation of the PI3K

pathway across doses. The RP2D of 400 mg QD was selected based on safety data. Significant target inhibition observed in tumor biopsies at this dose supported the selection.

Several genomic landscape studies of different tumor types have identified that *PIK3CB* is mutated or amplified in squamous cell lung [33], endometrial and head and neck cancers [34] and advanced prostate [27] and ovarian [35] cancers. Contrarily, in tumor types where activation of PI3Kα is more common, such as breast or colorectal cancer, genomic aberrations in *PIK3CB* are rare (<2%) [3,36]. We pursued retrospective tumor-targeted next-generation sequencing to explore putative predictive biomarkers of antitumor activity.

Of 48 samples analyzed, five (10%) had *PIK3CB* aberrations, including a somatic p.L1049R mutation. Additionally, two patients with increased *PIK3CB* copy number were also enrolled in the expansion phase. Among these seven patients, we observed one durable radiological partial response (on treatment for 14 months) and prolonged stabilizations of disease (on treatment for 24, 33, 34, and 57 weeks respectively). This association, albeit preliminary, is of particular interest in advanced prostate cancer, where molecular stratification for therapy selection remains an unmet medical need.

In conclusion, 400 mg QD continuous dosing was established as the RP2D for GSK2636771 based on dose-limiting toxicities. The safety profile of GSK2636771 400 mg QD, together with proof of target modulation and the preliminary association of clinical benefit with *PIK3CB* genomic aberrations, support the continued evaluation of this compound in Phase II clinical trials. The antitumor activity of GSK2636771 is being further studied as a single agent in molecularly-defined populations within the NCI-MATCH clinical trial, in combination with the androgen receptor antagonist enzalutamide (Xtandi®) in patients with CRPC, in combination with paclitaxel in gastric cancer and in combination with immunotherapy in melanoma.

METHODS

Preclinical studies

Cell lines and reagents

Breast (HCC1954 and MDA-MB-468) and prostate cancer (PC3, LNCAP, and DU-145) cell lines were obtained from ATCC. Cells were cultured in the appropriate medium supplemented with 10% fetal bovine serum (Sigma-Aldrich) at 37°C in humidified incubators under 5% CO_2. GSK2636771 was dissolved in dimethyl sulfoxide (DMSO) at a stock concentration of 20 mM.

Selectivity of GSK2636771 for PI3Kβ

Biochemical selectivity of GSK2636771 was tested using the PI3-Kinase HTRF™ Assay (EMD Millipore), as well as the entire panel of in-house kinase selectivity assays. In addition, GSK2636771 was tested at a single concentration (10 mM) against the 294 kinases in the Reaction Biology Corporation kinome panel. Affinity-enrichment based chemoproteomics using kinobeads was performed as described previously.[38] Briefly, 14 lipid and atypical kinases were enriched from a standard mixture of extracts derived from HeLa, K562, and Jurkat cells using a compound-derivatized bead matrix. The enriched proteins were identified by quantitative mass spectrometry analysis (MS/MS), enabling the simultaneous assessment of binding specificity and potency for all detected affinity-captured proteins.

Soft agar cell-viability assay

Cells were cultured in 96-well plates (5×10^3 cells/well) and treated with GSK2636771 (dose range: 30.7 mM–1.6 nM) for 6 days in soft agar media (bottom layer: 0.6% final concentration; top layer: 0.3% final concentration). Cell proliferation was measured using the alamarBlue® Cell Viability Assay (Thermo Fisher) according to the manufacturer's instructions. One cell plate was developed with alamarBlue®

reagent at the time of compound addition (T0 plate). Results were then expressed as a percentage of the T0 value (normalized to 100%) and plotted against the compound concentration after 6 days of treatment. The cellular response was determined by fitting the concentration response data using a 4-parameter curve fit equation and determining the concentration that inhibited 50% of the Y_{max}-Y_{min} window (EC_{50}).

Western blot analysis

Freshly harvested cancer cells were lysed with 1X cell lysis buffer (Cell Signaling Technology) containing protease and phosphatase inhibitors (Roche). Subsequently, 30–40 µg of protein was run on 4–12% Bis-Tris gels (Thermo Fisher), and protein was transferred onto nitrocellulose membranes (Thermo Fisher). Membranes were blocked for 1 hour using Odyssey® Blocking Buffer (LI-COR Biosciences), before immunoblotting using the following antibodies (all from Cell Signaling Technology): pAKT S473 (#4060), pAKT T308 (#13038), total AKT (#9272), pERK (#9101), total ERK (#4695), pS6 (#2211), total S6 (#2317), and PTEN (#9188). Western blots were processed using Odyssey® CLx Imaging System (LI-COR Biosciences).

In vivo studies

Female nude mice (Charles River Laboratories) were injected with 2.0 x 10^6 PC3 cells to establish subcutaneous PC3 tumor xenografts. Once tumors reached ~200–250 mm^3, mice were randomized (n=8/group) and treated with vehicle or GSK2636771 at 1, 3, 10, or 30 mg/kg by oral gavage for 21 days. Tumor volume measurements and body weights were collected twice weekly. For PK/PD studies, mice bearing PC3 tumor xenografts (n=3/group) were dosed once orally with either vehicle or GSK2636771 at 3 and 10 mg/kg for 1, 2, 4, 6, 8, 10, and 24 hours. Blood was collected and mixed 1:1 with water, and tumors were excised into two halves with one half flash frozen in liquid nitrogen for compound concentration determination

by the GSK Drug Metabolism and PK (DMPK) group. The other half of excised tumors was immediately processed using a sterile Medicon (BD Biosciences) in 1 mL Meso-Scale Discovery (MSD) lysis buffer containing protease and phosphatase inhibitors. Phospho and total AKT protein levels were measured using the MSD Phospho (Ser473)/Total AKT Whole Cell Lysate enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions. To measure glucose and insulin response, female nude mice (n=3/group) were dosed orally for three days with vehicle, 100 mg/kg GSK2636771, or 3 mg/kg GSK2126458 (a pan PI3K/mTOR inhibitor), then starved for 20 hours before receiving a final dose of compound followed by blood collection after 0, 0.5, 1, 2, and 4 hours. Compound concentrations were determined by the GSK DMPK group, glucose was measured using an ACCU-CHEK® Compact Plus glucose meter (Roche), and insulin was measured from plasma using an ALPCO Mouse Insulin ELISA Kit. All animal studies were conducted in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals and were reviewed by the Institutional Animal Care and Use Committee at GSK.

First-time-in-human study

Study design

The study followed a multi-stage design to minimize patient exposure to theoretically ineffective doses and prioritize acquisition of tumor tissue biopsies for PD analysis **(Supplementary Figure 2)**. Part 1 was a dose selection stage, to assess the PK of GSK2636771 following single-dose administration and determine the optimal starting dose for the Part 2. The primary objective of Part 1 was to establish a GSK2636771 dose that provided a median $AUC_{[0–24]}$ at steady-state of 10–50 µg*hr/mL). Part 2 was a dose-escalation stage utilizing a modified 3+3 design and allowing enrollment of additional patients for PD analysis of tumor biopsies. The primary objectives of Part 2 were to determine a recommended Phase II dose [RP2D], further characterize the PK

and PD of GSK2636771 after repeated daily dosing, and confirm the inhibition of PI3Kβ activity by GSK2636771 in tumor biopsies. Part 3 was an expansion cohort stage including patients with PTEN-deficient tumors and/or genomic *PIK3CB* genomic aberrations, to determine tumor responses to the RP2D of GSK2636771.

Clinical Trial Oversight

The study was designed by GSK representatives and study investigators. The research ethics committee at each participating site approved the study protocol. Data were collated and analyzed by GSK.

Trial population

Patients with advanced solid tumors progressing on standard therapy were enrolled after providing written consent and based on eligibility criteria. These included: age ≥18 years; Eastern Cooperative Oncology Group performance status 0–1; adequate organ function including renal function (based on blood creatinine and urine protein/creatinine ratio); and normal left-ventricular ejection fraction (LVEF). Patients receiving medication impacting platelet aggregation or with a baseline platelet-function defect were excluded. Full eligibility criteria can be found in the Supplementary Appendix.

For Parts 1 and 2, the target population were patients with PTEN-deficient tumors (determined by immunohistochemistry [IHC]) and one of the following primary tumor types: endometrial, ovarian, triple-negative breast cancer, CRPC, non-small cell lung cancer, glioblastoma, gastric adenocarcinoma, colorectal, head and neck squamous carcinoma and melanoma. In Part 3, the expansion cohorts included for patients with PTEN-deficient CRPC, colorectal cancer and/or genomic abnormalities (copy number gain or mutations) in *PIK3CB*.

Treatment, starting dose and dose-escalation

Treatment was administered orally as white gelatin capsules containing 10, 25 or 100 mg of GSK2636771. The starting dose in Part 1 was 25 mg QD, based on non-clinical toxicology studies predicting an $AUC_{(0–24)}$ in human subjects of 13 µg*hr/mL and a C_{max} of 0.85 µg/mL at steady-state. Part 2 followed a modified 3+3 design (**Supplementary Table 3**), starting at the selected dose from Part 1. DLTs were defined as any Grade 3/4 non-hematological drug-related toxicity (apart from Grade 3 rash, diarrhea, nausea, vomiting or mucositis that responds to treatment within 48 hours) occurring during the first 4-weeks of drug administration. Additionally, Grade 4 neutropenia lasting >5 days, Grade 4 anemia, Grade 4 thrombocytopenia (or Grade 3 with bleeding), an 8-fold increase in transaminases (over the upper limit of normal), a >20% decrease in LVEF, or any toxicity leading to >25% of the planned dose being missed, were also considered DLTs. Dose escalation was pursued until the maximum tolerated dose (MTD) was established, defined as the maximum dose level before DLTs were observed in ≥33% of patients.

Study Evaluations

Adverse events were recorded throughout the study, and graded based on Common Terminology Criteria for Adverse Events (CTCAE) v4.0, including monitoring of changes in renal function via blood and urine tests and other vital signs assessments. Cardiac evaluations (echocardiograms/multigated acquisition scans) were performed at baseline and bi-monthly during treatment. Response to therapy was assessed every 8 weeks by computed tomography/magnetic resonance imagery (and whole-body bone scintigraphy for patients with CRPC)[39]. Tumor markers were analyzed every 8 weeks if appropriate, according to tumor type.

Blood samples for PK analysis were collected at pre-specified time points after single dose administration (Parts 1 and 2) and then at Days 8, 15 and 22 during the first cycle of continuous treatment (Part 2).

Analyses of markers of target modulation (pSer473 AKT, pSer9 GSK3β and pThr421/Ser424 P70S6K) were undertaken on PRP from patients during the dose-escalation stage using MSD® electrochemiluminescent immunoassays validated to Good Clinical Practice standards. Changes in pSer473, pThr246 PRAS40, pSer235/236 and pThr308 were measured in tumor biopsies using immunohistochemistry (H-scores) at pre-treatment and Days 8–15 (2–4h post-dose).

Next generation sequencing and copy number analyses

Retrospective targeted next-generation sequencing (NGS) of archival or fresh tumor samples was performed if tissue was available. DNA was extracted using the GeneRead™ FFPE DNA Isolation kit (Qiagen, Hilden, Germany; cat#180134) and libraries prepared utilizing a customized sequencing panel (Qiagen GeneRead v2; Supplementary Table 2) including *PI3K/AKT* pathway genes, and sequencing was carried out on an Illumina Sequencer. Copy number variation was determined using Nanostring or quantitative polymerase chain reaction platforms. Background corrected, normalized values relative to a normal (diploid) control for 1–3 probes were used for each gene.

Functional characterization of the PIK3CB p.L1049R mutation *in vitro*

[to be completed]

Statistical considerations

Descriptive statistics were used to summarise safety data in all patients who received at least one dose of GSK2636771. All patients who underwent sampling were included in the PK analyses, which used descriptive statistics to summarize $AUC_{(0\text{-}t)}$, $AUC_{(0\text{-}24)}$, C_{max}, T_{max}, calculated using standard non-compartmental methods. Additionally,

$AUC_{(0-\infty)}$ and half-life were assessed after the single run-in dose. Tumor response rate was evaluated according to RECIST 1.1 criteria (37). The data were analyzed with Statistical Analysis Software (SAS®) version 9.2

Authors' Contributions

Conception and design: GG, JW, MM, RK, JSD

Development of methodology: GG, JW, MM, RK, JSD

Acquisition of data: JM, GG, HB, PD, DS, SKS, MM, S-WH, HCC, JPE, SS, Y-JB, JRI, JSD, H-TA

Analysis and interpretation of data: JM, GG, DS, SKS, JW, MM, RK, LY, JT, JSD

Writing, review and/or revision of the manuscript: All authors

Administrative, technical, or material support:

Study supervision:

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TABLES

Table 1. Baseline patient demographics and clinical characteristics

	Total (N=65)
Age, years	
Mean (SD)	59.7 (11.13)
Median (range)	62.0 (30–79)
Female, n (%)	26 (40)
Race, n (%)	
African American/African	2 (3)
Asian (Central/Southern)	1 (2)
Asian (Eastern)	13 (20)
Caucasian	48 (74)
Primary tumor type, n (%)	
Colon/rectum	23 (35)
Prostate	12 (18)
Gastric/GE junction	7 (11)
Breast (triple negative)	6 (9)
Ovary/fallopian tube	5 (8)
Endometrium/uterus	3 (5)
NSCLC	3 (5)
CNS	2 (3)
Head and neck	2 (3)
Melanoma	1 (2)
Cervix	1 (2)

CNS, central nervous system; GE, gastroesophageal; NSCLC, non-small cell lung cancer; SD, standard deviation

Table 2. Summary of AEs occurring in >20% of all patients, treatment-related AEs and SAEs

Preferred term, n (%)	Dose selection cohort	Dose escalation cohort			PD cohort	Expansion cohort	Total
	25 mg n=3	25–350 mg n=23	400 mg n=6	500 mg n=4	50–350 mg n=17	400 mg n=12	(N=65)
Any AE (any Grade)	3 (100)	23 (100)	6 (100)	4 (100)	17 (100)	12 (100)	65 (100)
Diarrhea	1 (33)	11 (48)	1 (17)	1 (25)	9 (53)	8 (67)	31 (48)
Nausea	2 (67)	9 (39)	2 (33)	1 (25)	7 (41)	5 (42)	26 (40)
Vomiting	1 (33)	9 (39)	1 (17)	1 (25)	6 (35)	2 (17)	20 (31)
Fatigue	1 (33)	8 (35)	1 (17)	2 (50)	2 (12)	2 (17)	16 (25)
Anemia	0	7 (30)	0	0	5 (29)	3 (25)	15 (23)
Abdominal pain	2 (67)	7 (30)	1 (17)	1 (25)	2 (12)	1 (8)	14 (22)
Decreased appetite	0	4 (17)	1 (17)	1 (25)	6 (35)	2 (17)	14 (22)
Headache	1 (33)	6 (26)	0	0	2 (12)	4 (33)	13 (20)
Treatment-related AEs	3 (100)	22 (96)	5 (83)	4 (100)	15 (88)	11 (92)	60 (92)
Any SAE	1 (33)	7 (30)	0	1 (25)	9 (53)	6 (50)	24 (37)

Fatal SAEs	0	0	0	0	0	1 (8)	1 (2)

AE, adverse event; PD, pharmacodynamics; SAE, serious AE

Table 3. PK parameters following repeated daily oral dosing of GSK2636771 (PK population)

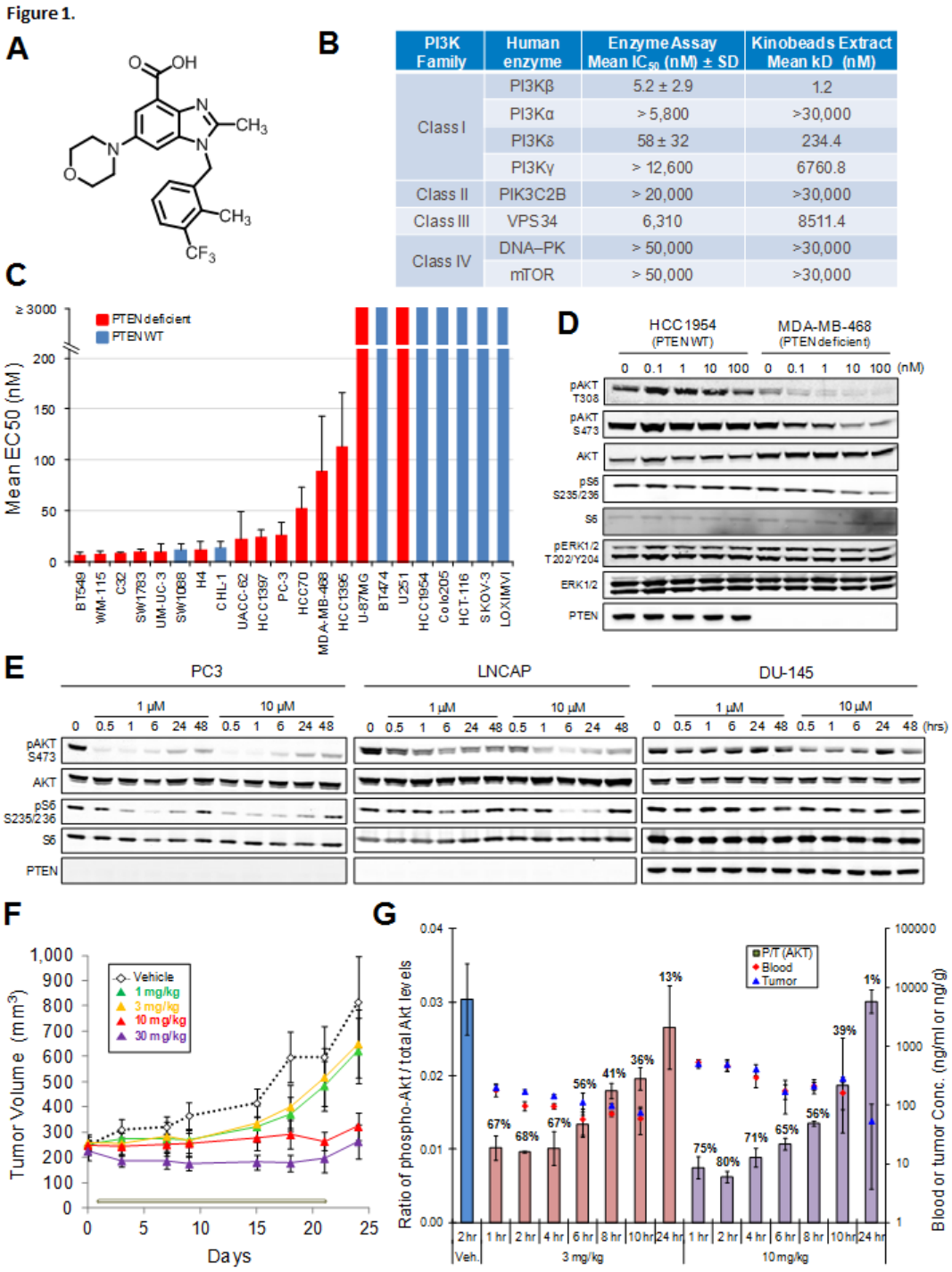
Preferred term, n (%)	**Dose selection cohort**	**Dose escalation cohort**						
	25 mg n=3	**25 mg n=6**	**50 mg n=4**	**100 mg n=3**	**200 mg n=3**	**350 mg n=7**	**400 mg n=6**	**500 mg n=4**
C_{max}, ng/mL*	n=2	n=5	n=4	n=3	n=3	n=4	n=6	n=2
	1459 (13)	1770 (65)	2336 (43)	2882 (85)	13175 (6)	16452 (9)	15078 (55)	29530 (71)
T_{max}, h, median (range)	n=2	n=5	n=4	n=3	n=3	n=4	n=6	n=2
	8.96	4.05	4.05	3.25	23.9	3.60	2.01	6.33
	(8.03, 9.88)	(3.00, 6.07)	(4.03, 6.00)	(3.10, 8.02)	(3.22, 23.92)	(2.03, 8.85)	(1.02, 5.80)	(2.58, 10.08)
$AUC_{(0–τ)}$, h·ng/mL*	n=2	n=4	n=4	n=2	n=1	n=4	n=5	n=2
	26181 (23)	28951 (77)	33052 (31)	30866 (136)	189479	282665 (25)	205014 (41)	485325 (70)

*Data presented as geometric mean (CVb%)

$AUC_{(0–τ)}$, area under the time concentration-time curve over the dosing interval; C_{max}, maximum observed plasma concentration; T_{max}, time to reach C_{max}

FIGURE LEGENDS

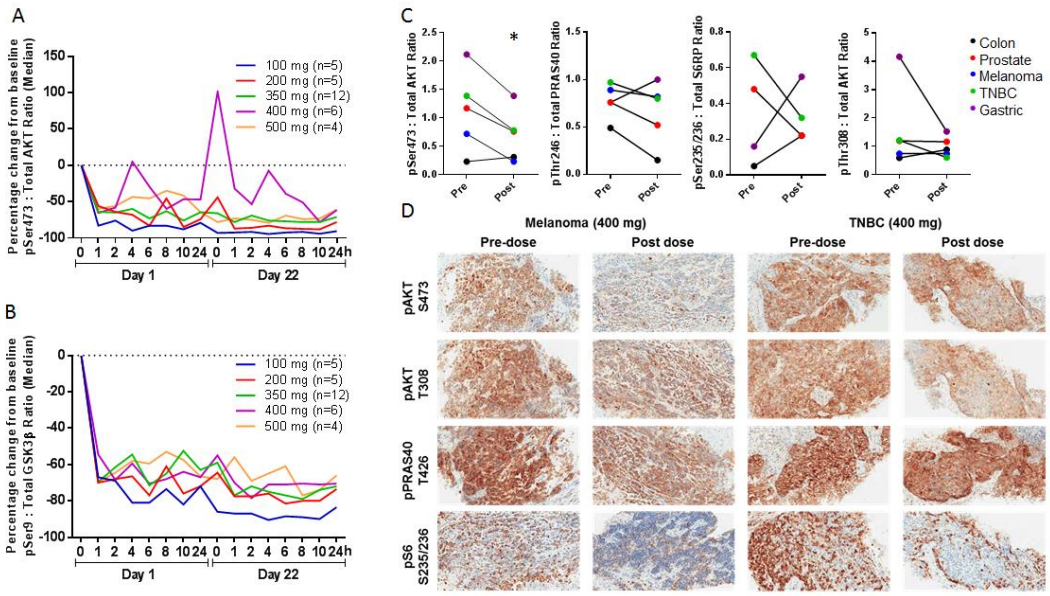
Figure 1. GSK2636771 is a potent, selective inhibitor of PI3Kβ that exhibits antitumor activity in PTEN-deficient cancers.



A, chemical structure of GSK2636771. **B,** The selectivity of GSK2636771 for PI3Kβ against other PI3K isoforms and PI3K family members was tested in a biochemical

activity assay (left column) and used a cancer cell lysate-based chemoproteomics approach to measure binding affinity (right column). **C,** Anchorage independent tumor cell growth was measured after 6 days of GSK2636771 treatment (dose range: 0.16–3.07 µM) comparing PTEN wild-type to PTEN-deficient cells. Error bars correspond to standard deviation. **D,** HCC1954 and MDA-MB-468 breast cancer cells were treated with increasing concentrations of GSK2636771 for 24 hours, and lysates were probed by Western blot using the indicated antibodies. **E,** PC3, LNCAP, and DU-145 prostate cancer cells were treated with 1 or 10 µM GSK2636771 for up to 48 hours and probed with the indicated antibodies. **F,** Mice bearing subcutaneous PC3 tumor xenografts (n=8/group) were treated with vehicle or GSK2636771 (1, 3 or 10 mg/kg) once daily by oral gavage for 21 days, and tumor volumes were assessed. Error bars correspond to standard error of the mean. **G,** PK/PD relationship of GSK2636771 was tested in mice bearing PC3 tumor xenografts (n=3/group) dosed once orally with either vehicle, 3 mg/kg or 10 mg/kg of GSK2636771. Tumors and blood samples were harvested at the indicated time points to measure plasma compound concentration and the ratio of phospho AKT to total AKT in tumors using enzyme-linked immunosorbent assays. The numbers above the bars indicate percent inhibition of pAKT relative to vehicle-treated tumors. Error bars correspond to standard deviation.

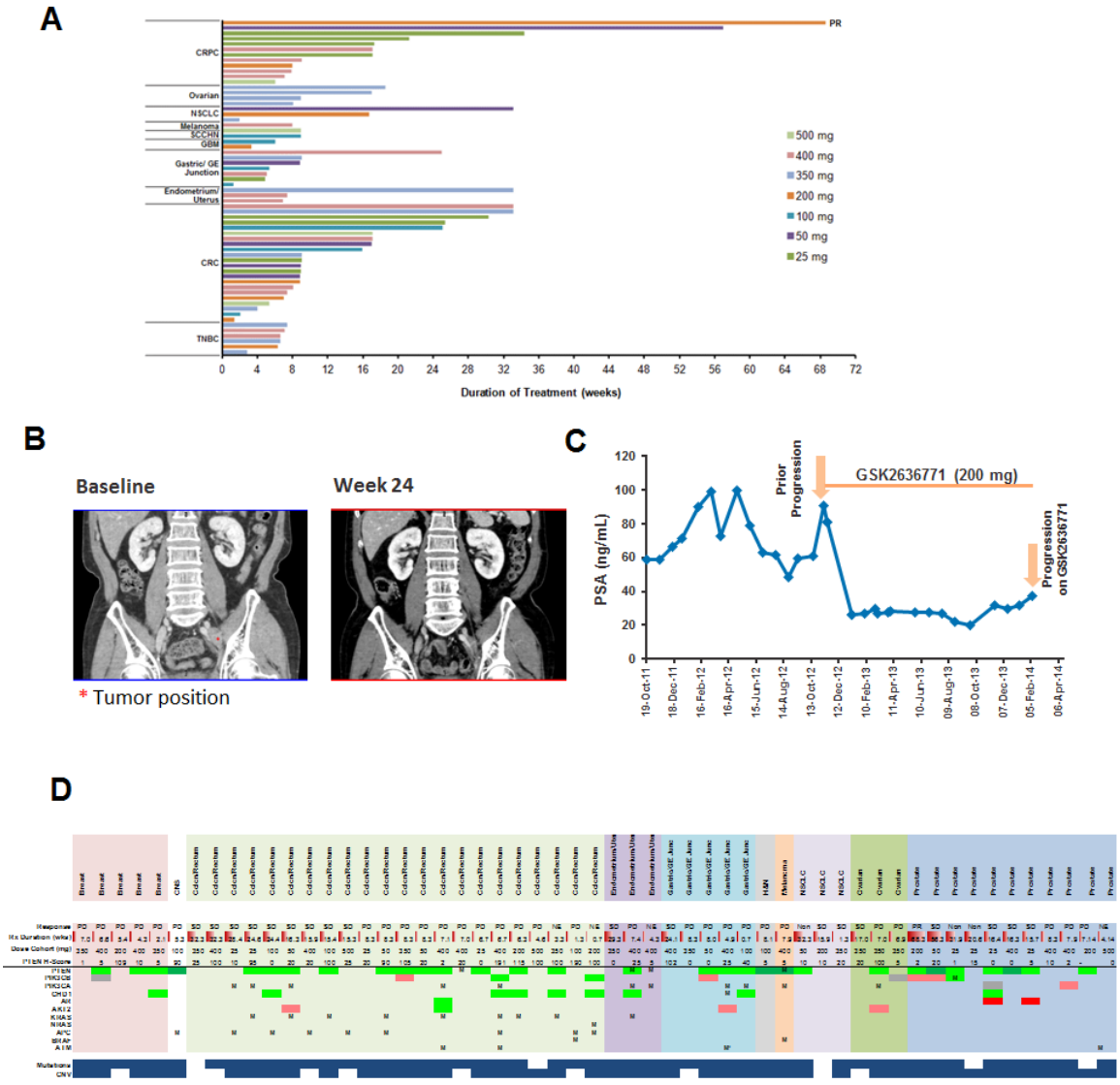
Figure 2. GSK2636771 inhibits PI3K signaling at doses of 100–500 mg QD

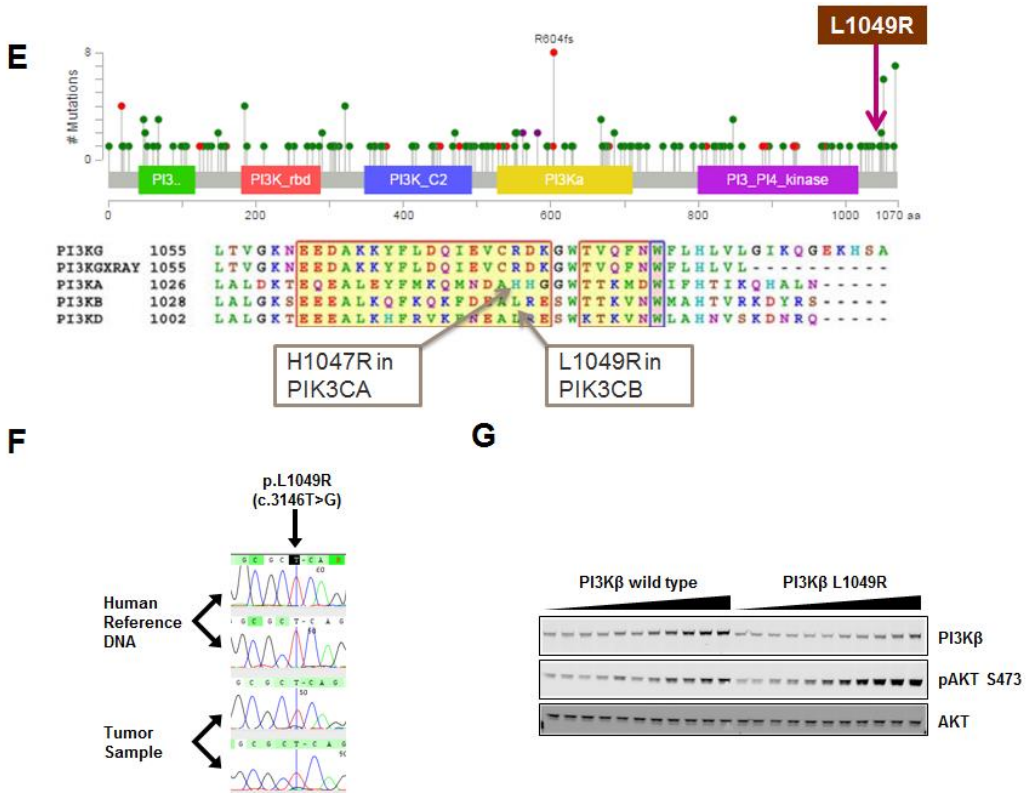


Median values of pAKT/total AKT ratio (**A**) and pGSK3β/total GSK3β (**B**) were measured in platelet rich plasma on Day 1, Cycle 1, using Meso Scale Discovery electrochemiluminescent assay; **C,** changes in pSer473, pThr246 PRAS40, pSer235/236 and pThr308 were measured in tumor biopsies using immunohistochemistry (H-scores) at pre-treatment and Days 8–15 (2–4h post-dose); **D,** representative photomicrographs (20x magnification) of immunohistochemistry staining showing tumor PD effects of p-AKT (S473, T308), p-PRAS40 (T246) and pS6RP (S235/236) for two patients treated with GSK2636771 400 mg QD (melanoma and TNBC). Biopsies were collected 2–4 hours post-dose between Day 8 and 15

AKT, protein kinase B; PD, pharmacodynamic; PI3K, phosphoinositide 3 kinase; QD, once daily; TNBC, triple negative brain cancer

Figure 3. Treatment duration (all treated population), partial response in one patient with CRPC, retrospective archival tumor biopsy analysis for key mutation and copy number events (all treated population), and functional analysis of the PIK3CB p.L1049R mutation





A, Treatment duration showed that one patient with CRPC had a PR, 17 patients had SD, eight of whom received GSK2636771 treatment for ≥6 months; **B,** The partial response was observed at Week 24 in the patient with CRPC, and was accompanied by a 78% reduction in PSA levels (**C**), which was durable with progression after 16 months of treatment; **D,** Data for 55 patients whose tumor tissue was analyzed retrospectively for somatic mutations and copy number alterations: one copy loss (light green); two copy loss (dark green); gain (light red); amplification (dark red); coding mutations (M); non-coding mutations (M*); no data (grey). CNV was determined based on Nanostring or qPCR platforms; **E**, The *PI3Kβ* L1049R mutation is homologous to H1047R in *PI3KCA*, as reported in a patient with glioblastoma (www.cbioportal.org); **F,** Confirmation of the presence of the *PI3Kβ* p.L1049R mutation was achieved using Sanger sequencing; **G,** PC3 cells were transduced with pHTBBV1.1 (using baculovirus gene transfer into mammalian cells) expressing wt *PI3Kβ* or the p.L1049R mutant at a range of multiplicity of infections, and analyzed using Western blot.

AKT, protein kinase B; APC, Adenomatous polyposis coli; AR, androgen receptor; ATM, ataxia telangiectasia mutated; BRAF, v-raf murine sarcoma viral oncogene homolog B; CHD1, chromodomain helicase DNA binding protein 1; CNS, central nervous system; CNV, copy number variation; CRPC, castration-resistant prostate cancer; DNA, deoxyribonucleic acid; GE, gastrointestinal; GMB, glioblastoma; H&N, head and neck; KRAS, V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; NE/Non, not evaluable; NRAS, neuroblastoma rat sarcoma viral oncogene homolog; NSCLC, non-small cell lung cancer; PI3K, phosphoinositide-3-kinase; qPCR, quantitative polymerase chain reaction; PD, progressive disease; *PI3K*, phosphoinositide-3-kinase; PR, partial response; PSA, prostate-specific antigen; PTEN, phosphatase and tensin homolog; SD, stable disease; wt, wild type

References

1. Liu P, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. *Nature reviews Drug discovery* 2009; **8**(8): 627-44.
2. Koboldt DC FR, McLellan MD, Schmidt H, Kalicki-Veizer J, McMichael JF, et al. Comprehensive molecular portraits of human breast tumours. *Nature* 2012; **490**(7418): 61-70.
3. Muzny DM BM, Chang K, Dinh HH, Drummond JA, Fowler G, et al. . Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012; **487**(7407): 330-7.
4. Di Cristofano A, Pandolfi PP. The multiple roles of PTEN in tumor suppression. *Cell* 2000; **100**(4): 387-90.
5. Samuels Y, Wang Z, Bardelli A, et al. High frequency of mutations of the PIK3CA gene in human cancers. *Science (New York, NY)* 2004; **304**(5670): 554.
6. Courtney KD, Corcoran RB, Engelman JA. The PI3K pathway as drug target in human cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2010; **28**(6): 1075-83.
7. Rodon J, Dienstmann R, Serra V, Tabernero J. Development of PI3K inhibitors: lessons learned from early clinical trials. *Nature reviews Clinical oncology* 2013; **10**(3): 143-53.
8. Chandarlapaty S, Sawai A, Scaltriti M, et al. AKT inhibition relieves feedback suppression of receptor tyrosine kinase expression and activity. *Cancer cell* 2011; **19**(1): 58-71.
9. Stratikopoulos EE, Dendy M, Szabolcs M, et al. Kinase and BET Inhibitors Together Clamp Inhibition of PI3K Signaling and Overcome Resistance to Therapy. *Cancer cell* 2015; **27**(6): 837-51.
10. Ihle NT, Lemos R, Jr., Wipf P, et al. Mutations in the phosphatidylinositol-3-kinase pathway predict for antitumor activity of the inhibitor PX-866 whereas oncogenic Ras is a dominant predictor for resistance. *Cancer research* 2009; **69**(1): 143-50.
11. Busaidy NL, Farooki A, Dowlati A, et al. Management of metabolic effects associated with anticancer agents targeting the PI3K-Akt-mTOR pathway. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2012; **30**(23): 2919-28.
12. Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nature reviews Genetics* 2006; **7**(8): 606-19.
13. Brachmann SM, 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. *Molecular cancer therapeutics* 2012; **11**(8): 1747-57.
14. Jia S, Liu Z, Zhang S, et al. Essential roles of PI(3)K-p110beta in cell growth, metabolism and tumorigenesis. *Nature* 2008; **454**(7205): 776-9.
15. Ni J, Liu Q, Xie S, et al. Functional characterization of an isoform-selective inhibitor of PI3K-p110beta as a potential anticancer agent. *Cancer discovery* 2012; **2**(5): 425-33.
16. Schwartz S, Wongvipat J, Trigwell CB, et al. Feedback suppression of PI3Kalpha signaling in PTEN-mutated tumors is relieved by selective inhibition of PI3Kbeta. *Cancer cell* 2015; **27**(1): 109-22.
17. Wee S, Wiederschain D, Maira SM, et al. PTEN-deficient cancers depend on PIK3CB. *Proceedings of the National Academy of Sciences of the United States of America* 2008; **105**(35): 13057-62.

18. Foukas LC, Claret M, Pearce W, et al. Critical role for the p110alpha phosphoinositide-3-OH kinase in growth and metabolic regulation. *Nature* 2006; **441**(7091): 366-70.
19. Carver BS, Chapinski C, Wongvipat J, et al. Reciprocal feedback regulation of PI3K and androgen receptor signaling in PTEN-deficient prostate cancer. *Cancer cell* 2011; **19**(5): 575-86.
20. Ferraldeschi R, Nava Rodrigues D, Riisnaes R, et al. PTEN protein loss and clinical outcome from castration-resistant prostate cancer treated with abiraterone acetate. *European urology* 2015; **67**(4): 795-802.
21. Reid AH, Attard G, Danila DC, et al. Significant and sustained antitumor activity in post-docetaxel, castration-resistant prostate cancer with the CYP17 inhibitor abiraterone acetate. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2010; **28**(9): 1489-95.
22. Bosch A, Li Z, Bergamaschi A, et al. PI3K inhibition results in enhanced estrogen receptor function and dependence in hormone receptor-positive breast cancer. *Science translational medicine* 2015; **7**(283): 283ra51.
23. Crowder RJ, Phommaly C, Tao Y, et al. PIK3CA and PIK3CB inhibition produce synthetic lethality when combined with estrogen deprivation in estrogen receptor-positive breast cancer. *Cancer research* 2009; **69**(9): 3955-62.
24. Serra V, Scaltriti M, Prudkin L, et al. PI3K inhibition results in enhanced HER signaling and acquired ERK dependency in HER2-overexpressing breast cancer. *Oncogene* 2011; **30**(22): 2547-57.
25. Zhang Y, Moerkens M, Ramaiahgari S, et al. Elevated insulin-like growth factor 1 receptor signaling induces antiestrogen resistance through the MAPK/ERK and PI3K/Akt signaling routes. *Breast cancer research : BCR* 2011; **13**(3): R52.
26. Dienstmann R, Rodon J, Serra V, Tabernero J. Picking the point of inhibition: a comparative review of PI3K/AKT/mTOR pathway inhibitors. *Molecular cancer therapeutics* 2014; **13**(5): 1021-31.
27. Brennan CW, Verhaak RG, McKenna A, et al. The somatic genomic landscape of glioblastoma. *Cell* 2013; **155**(2): 462-77.
28. Nakanishi Y, Walter K, Spoerke JM, et al. Activating Mutations in PIK3CB Confer Resistance to PI3K Inhibition and Define a Novel Oncogenic Role for p110beta. *Cancer research* 2016; **76**(5): 1193-203.
29. Garcia A, Kim S, Bhavaraju K, Schoenwaelder SM, Kunapuli SP. Role of phosphoinositide 3-kinase beta in platelet aggregation and thromboxane A2 generation mediated by Gi signalling pathways. *The Biochemical journal* 2010; **429**(2): 369-77.
30. Hammerman PS LM, Voet D, Jing R, Cibulskis K, Sivachenko A, et al. . Comprehensive genomic characterization of squamous cell lung cancers. *Nature* 2012; **489**(7417): 519-25.
31. Lawrence MS SC, Lichtenstein L, Cibulskis K, Lander E, Gabriel SB, et al. Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature* 2015; **517**(7536): 576-82.
32. Robinson D, Van Allen EM, Wu YM, et al. Integrative clinical genomics of advanced prostate cancer. *Cell* 2015; **161**(5): 1215-28.
33. Bell D BA, Birrer M, Chien J, Cramer DW, Dao F, et al. Integrated genomic analyses of ovarian carcinoma. *Nature* 2011; **474**(7353): 609-15.
34. Ciriello G, Gatza ML, Beck AH, et al. Comprehensive Molecular Portraits of Invasive Lobular Breast Cancer. *Cell* 2015; **163**(2): 506-19.
35. Abeshouse A AJ, Akbani R, Ally A, Amin S, Andry CD, et al. The Molecular Taxonomy of Primary Prostate Cancer. *Cell* 2015; **163**(4): 1011-25.
36. Bantscheff M, Eberhard D, Abraham Y, et al. Quantitative chemical proteomics reveals mechanisms of action of clinical ABL kinase inhibitors. *Nat Biotechnol* 2007; **25**(9): 1035-44.

37. Scher HI, Halabi S, Tannock I, et al. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2008; **26**(7): 1148-59.