A first-time-in-human study of GSK2636771, a phosphoinositide 3 kinase beta-selective inhibitor, in patients with advanced solid tumors

Joaquin Mateo[1], Gopinath Ganji[2], Charlotte Lemech[3], Howard A. Burris[4], Sae-Won Han[5], Karen Swales[1], Shaun Decordova[1], M. Phillip DeYoung[2], Deborah A. Smith[6], Shanker Kalyana-Sundaram[2], Jiuhua Wu[7], Monica Motwani[8], Rakesh Kumar[9], Jerry M. Tolson[2], Sun Young Rha[10], Hyun Cheol Chung[10], Joseph P. Eder[11], Sunil Sharma[12], Yung-Jue Bang[5], Jeffrey R. Infante[4], Li Yan[2*], Johann S. de Bono[1*], Hendrik-Tobias Arkenau[3]

[1]Drug Development Unit, The Institute of Cancer Research and The Royal Marsden NHS Foundation Trust, London, UK; [2]GSK, 1250 S Collegeville Rd, Collegeville, PA, USA; [3]Sarah Cannon Research Institute UK, University College London Cancer Centre, London, UK; [4]Sarah Cannon Research Institute/Tennessee Oncology, Nashville, TN, USA; [5]Seoul National University Hospital, Seoul National University College of Medicine Seoul, South Korea; [6]PAREXEL International, 2560 Meridian Parkway, Durham, NC, USA; [7]Biostat Consulting, Inc., Portage, MI, USA; [8]AbbVie Ltd, Translational Oncology & Precision Medicine, Chicago, IL, USA; [9]MedImmune, Gaithersburg, MD, USA; [10]Yonsei Cancer Center, Yonsei University College of Medicine, Seoul, South Korea; [11]Yale Cancer Center, New Haven, CT, USA; [12]University of Utah Huntsman Cancer Institute, Salt Lake City, UT, USA

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***Corresponding authors:**

Professor Johann S. de Bono, MB ChB, MSc, FRCP, PhD, FMedSci

Professor of Experimental Cancer Medicine

Division of Clinical Studies, The Institute of Cancer Research

Drug Development Unit, The Royal Marsden NHS Foundation Trust

Downs Road

Sutton

Surrey SM2 5PT

UK

Tel: +44 (0)2087224028

Fax: +44 (0)2086427979

Email: johann.de-bono@icr.ac.uk

Dr Li Yan, MD, PhD

VP & Head Unit Physician

GSK Oncology

GSK

1250 S Collegeville Rd

Collegeville

PA, USA

Tel: +16109175388

Fax: +16109174100

Email: li.1.yan@gsk.com

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Statement of translational relevance

The PI3K/AKT pathway is commonly activated in several tumor types. Selective targeting of p110β could result in successful pathway inhibition while avoiding the on and off target effects of pan-PI3K inhibitors. We present preclinical studies conducted to characterize the effect and define the optimal target population for development of GSK2636771, a selective p110β inhibitor, and results from a first-time-in-human clinical trial study indicating that GSK2636771 can be active in PTEN-deficient and/or *PIK3CB*-aberrant advanced solid tumors. Further Phase II clinical studies of GSK2636771 in combination with other agents, including androgen receptor antagonists, are currently underway based on these data.

Abstract

Background: The phosphoinositide 3 kinase (PI3K)/protein kinase B (AKT) pathway is commonly activated in several tumor types. Selective targeting of p110β could result in successful pathway inhibition while avoiding the on and off target effects of pan-PI3K inhibitors. GSK2636771 is a potent, orally bioavailable, adenosine triphosphate-competitive, selective inhibitor of PI3Kβ.

Methods: We evaluated the safety, pharmacokinetics, pharmacodynamics and antitumor activity of GSK2636771 to define the recommended Phase II dose (RP2D). During the dose-selection and dose-escalation stages (Parts 1 and 2), patients with phosphatase and tensin homolog (*PTEN*)-deficient advanced solid tumors received escalating doses of GSK2636771 (25–500 mg once daily [QD]) using a modified 3+3 design to determine the RP2D; tumor type-specific expansion cohorts (Part 3) were implemented to further assess tumor responses at the RP2D.

Results: A total of 65 patients were enrolled; dose-limiting toxicities were hypophosphatemia and hypocalcemia. Adverse events included diarrhea (48%), nausea (40%), and vomiting (31%). Single- and repeat-dose exposure increased generally dose proportionally. GSK2636771 400 mg QD was the RP2D. Phospho/total AKT ratio decreased with GSK2636771 in tumor and surrogate tissue. A castrate-resistant prostate cancer (CRPC) patient harboring *PIK3CB* amplification had a partial response for over a year; an additional 10 patients derived durable (≥24 weeks) clinical benefit, including 2 other patients with CRPC with *PIK3CB* alterations (≥34 weeks). GSK2636771 400 mg QD oral induced sufficient exposure and target inhibition with a manageable safety profile.

Conclusions: Genomic aberrations of *PIK3CB* may be associated with clinical benefit from GSK2636771.

Introduction

Activation of phosphoinositide 3 kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signaling (1), most commonly by activating mutations of *PI3K/AKT* family members or loss of phosphatase and tensin homolog (PTEN) phosphatase function, contributes to carcinogenesis of many malignancies (2-5). Inhibition of PI3K signaling has been challenging therapeutically, and inhibitors of PI3K have so far 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 inhibitors, resulting in a plethora of PI3K-related and off-target toxicities that limit administration of clinically active doses for long enough (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 toxicities seen with pan-PI3K inhibitors (13).

Loss of PTEN deleted on chromosome ten function is common 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 primarily PI3K pathway activation, cell growth and survival in PTEN-deficient tumor cells (14-17).

We hypothesize that highly selective PI3Kβ inhibition would have utility in PTEN-deficient cancers, whilst avoiding toxicities associated with inhibition of other PI3K isoforms (18), in particular skin rash, hyperglycemia and diarrhea, or other off-target effects. This strategy would be expected to maximize therapeutic efficacy by enabling administration of appropriate doses and rational drug combinations with other agents, such as androgen receptor 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-competitive, selective inhibitor of PI3Kβ with an apparent Ki value of 0.89 nM (IC_{50} = 5.2 nM), >900-fold selectivity over p110α and p110γ, and >10-fold selectivity over p110δ isoforms, while sparing other PI3K superfamily kinases (Figure 1B). While pan-PI3K inhibitors have been tested in clinical trials, to our knowledge, this is the first study of a truly selective inhibitor of PI3Kβ, which confers the advantage of avoiding on- and off-target toxicities associated with pan-PI3K inhibitors.

Here we present preclinical data characterizing the selectivity of GSK2636771 in cell line and murine xenograft models, together with the results of a dose-finding, first-time-in-human study of GSK2636771 monotherapy in patients with PTEN-deficient or *PIK3CB* genomically-altered advanced solid tumors. The aim of this first-time-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 identify any alterations as putative predictive biomarkers of antitumor response to determine the optimal target population.

Materials and Methods

Preclinical studies

Cell lines and reagents

Cell lines were obtained from ATCC (Manassas, Virginia, USA), cultured in the appropriate medium supplemented with 10% fetal bovine serum (Sigma−Aldrich, St. Louis, Missouri, USA) at 37°C in humidified incubators under 5% carbon dioxide, and passaged no greater than 20 times. The cell lines were authenticated by short tandem repeat (STR) profiling and tested for *Mycoplasma* upon receipt using the ATCC Universal Mycoplasma Detection Kit. GSK2636771 was dissolved in dimethyl sulfoxide 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, Billerica, Massachusetts, USA), as well as the entire panel of GSK in-house kinase selectivity assays. Affinity-enrichment based chemoproteomics using kinobeads was performed as described previously (26). 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, 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 μM–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, Waltham, Massachusetts, USA) 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

HCC1954 and MDA-MB-468 breast cancer cells were treated with increasing concentrations of GSK2636771 for 24 hours. PC3 prostate cancer cells were treated with 1 or 10 μM GSK2636771 for up to 48 hours. Cells were lysed with 1X cell lysis buffer (Cell Signaling Technology, Danvers, Massachusetts, USA) containing protease and phosphatase inhibitors (Roche, Basel, Switzerland). Subsequently, 30–40 μg of protein was run on 4–12% Bis-Tris

gels (Thermo Fisher, Waltham, Massachusetts, USA), and protein was transferred onto nitrocellulose membranes (Thermo Fisher, Waltham, Massachusetts, USA). Membranes were blocked for 1 hour using Odyssey® Blocking Buffer (LI-COR Biosciences, Lincoln, Nebraska, USA), before immunoblotting using the following antibodies (all from Cell Signaling Technology, Danvers, Massachusetts, USA): pAKT Ser473 (#4060), pAKT Thr308 (#13038), total AKT (#9272), pERK (#9101), total ERK (#4695), pS6 (#2211), total S6 (#2317), PTEN (#9188), and p100β (#3011). Western blots were processed using Odyssey® CLx Imaging System (LI-COR Biosciences, Lincoln, Nebraska, USA).

In vivo studies

Female nude mice (Charles River Laboratories, Wilmington, Massachusetts, USA) were injected with 2.0×10^6 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, San Jose, California, USA) 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 3 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, Basel, Switzerland), 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 maximize the number of patients receiving potentially active doses and prioritize acquisition of tumor tissue biopsies for PD analysis (Supplementary Figure 1). Part 1 was a dose selection stage, to assess the PK of GSK2636771 following single-dose administration and determine the optimal starting dose for Part 2. The primary objective of Part 1 was to establish a GSK2636771 dose that provided a median area under the concentration-time curve 0 to 24 hours ($AUC_{[0–24]}$) 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, castrate-resistant prostate 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 patients with PTEN-deficient CRPC, colorectal cancer and/or genomic abnormalities (copy number gain or mutations) in *PIK3CB*. For eligibility purposes, PTEN assessments during the dose-escalation stage of the trial were performed at either the local laboratory of the investigator sites or at a central laboratory (Ventana Medical Systems, Tucson, Arizona, USA). In the expansion phase of the study, all samples were tested at the central laboratory (Ventana Medical Systems, Tucson, Arizona, USA) prior to enrollment and loss of PTEN function was defined as an H-score ≤30, with a maximum of 30% of cells at a 1+ staining intensity. A rabbit monoclonal anti-PTEN antibody (clone D4.3, catalog no. 9188, Cell Signaling Technologies, Danvers, Massachusetts, USA) was used for PTEN IHC staining.

Treatment, starting dose, and dose-escalation

Treatment was administered orally as white gelatin capsules containing 10, 25, or 100 mg of GSK2636771. Based on non-clinical toxicology studies predicting an $AUC_{[0–24]}$ in human subjects of 13 µg*hr/mL and a maximum observed plasma concentration (C_{max}) of 0.85 µg/mL at steady state, the starting dose in Part 1 was 25 mg. This was <1/20 of the

dose estimated with FDA recommendations for starting doses based on the highest non-severely toxic dose (HNSTD) of 100 mg/kg/day, which was also the no observed adverse effect level (NOAEL) in canine studies. Part 2 followed a modified 3+3 design (Supplementary Table 1), starting at the selected dose from Part 1. Dose-limiting toxicities (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 was established, defined as the maximum dose level before DLTs were observed in ≥33% of patients.

Study evaluations

Adverse events (AEs) were recorded throughout the study, and graded based on Common Terminology Criteria for Adverse Events 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) (27). Tumor markers were analyzed every 8 weeks if appropriate, according to tumor type.

Blood samples for PK analysis were collected ≤1 hour pre-dose and 0.5, 1, 2, 3, 4, 6, 8, 10, 24, 48, and 72 hours after single dose administration (Parts 1 and 2) and then ≤1 hour pre-dose on Days 8 and 15 and ≤1 hour pre-dose and 0.5, 1, 2, 3, 4, 6, 8, 10, and 24 hours after administration on Day 22 during the first cycle of continuous treatment (Part 2). Blood

samples at ≤1 hour pre-dose, 1–2 hours, 3–4 hours, 6–8 hours, and 22–26 hours post dose on Day 22 were collected in Part 3.

Analyses of markers of target modulation (pSer473 AKT, pSer9 GSK3β and pThr421/Ser424 P70S6K) were undertaken on platelet rich plasma (PRP) from patients during the dose-escalation stage using MSDelectrochemiluminescent immunoassays validated to Good Clinical Practice standards. Changes in pSer473 AKT, pThr246 PRAS40, pSer235/236 S6RP and pThr308 AKT were measured in tumor biopsies using IHC (H-scores) at pretreatment and Days 8–15 (2–4 hours post dose).

Next-generation sequencing and copy number analyses

Retrospective targeted next-generation sequencing of archival or fresh tumor samples was performed if tissue was available. DNA was extracted using the GeneRead™ formalin-fixed, paraffin-embedded 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*

BacMam vectors (pHTBV1.1) containing human wild-type (WT) p110β or mutant p110β (L1049R) were obtained from the GSK plasmid repository. Viral particles were generated and added into 6-well plates at a range of 0 to 500 multiplicity of infection. PC3 cells were then plated in the wells (1.0×10^6 cells/well) and allowed to incubate overnight. The media containing viral particles was removed and replaced with media lacking serum for 10 hours. PC3 cells were then lysed for Western blot analysis.

Statistical considerations

The number of subjects in Parts 1 and 2 was dependent on the number of subjects enrolled to select a starting dose, characterize individual cohorts, and explore the PD profile. Planned enrollment for Part 3 included a minimum of 12 and maximum of 20 subjects in each Tumor-Specific Expansion Cohort

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}, time to reach C_{max} (T_{max}), calculated using standard non-compartmental methods. Additionally, $AUC_{(0\text{-}\infty)}$ and half-life were assessed after the single run-in dose. Tumor response rate was evaluated according to Response Evaluation Criteria In Solid Tumors (RECIST) 1.1 criteria (28). The data were analyzed with Statistical Analysis Software (SAS®, Cary, North Carolina, USA) v9.2.

Results

Preclinical studies

To determine the effects of selectively inhibiting PI3Kβ activity on tumor cell growth and pathway signaling, a series of experiments was performed with GSK2636771 to compare its effects in PTEN-deficient and PTEN WT tumor cells. GSK2636771 primarily inhibited the growth of PTEN-deficient cancer cells in a cell line panel spanning multiple tumor types (Figure 1C). Inhibition of AKT and ribosomal S6 kinase phosphorylation was observed in a concentration- and time-dependent manner mainly in PTEN-deficient cells (Figures 1D and 1E and Supplementary Figure 2). GSK2636771 had no effect on mitogen-activated protein kinase (MAPK) signaling, as evidenced by measurement of extracellular signal-regulated kinase (ERK) phosphorylation (Figure 1D). When administered orally in mice bearing PC-3 prostate tumor xenografts, GSK2636771 resulted in 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/mTOR inhibitor, GSK2126458 (Supplementary Figure 3).

First-time-in-human clinical study

Patients and administered treatments

Overall, 65 patients were enrolled and received at least one dose of study medication. Data from 3 patients in Part 1 (dose selection), 50 in Part 2 (dose-escalation and additional PD exploratory cohorts), and 10 as part of the expansion cohorts in Part 3 made up a cohort of 63 patients. Data from the last 2 patients with known *PIK3CB* genomic alterations made up the overall trial population of 65, but were analyzed separately for some parameters and were not included in the PK-PD analyses. Baseline patient demographics and characteristics for the entire population are summarized in Table 1. Briefly, the median age of the study population was 62 years (range 30–79), 26 (40%) patients were female, and the most common tumor types were colorectal (n=23, 35%) and prostate cancers (n=12, 18%). All patients had received at least one previous anticancer treatment; 37 (57%) had received >4 anticancer treatments. In total, seven dose levels (25–500 mg QD) were investigated. Median time on treatment was 55 days (range 5–478).

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 $AUC_{[0–24]}$ was 15.7 μg*hr/mL, which was within the prespecified target range of 10 to 50 μg*hr/mL. Consequently, 25 mg QD was selected as the initial dose for the dose-escalation stage.

Safety, tolerability, and DLTs

No 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 first to third week of continuous treatment. One patient also experienced a Grade 1 creatinine elevation.

These toxicities, indicative of potential renal tubular damage, resolved after GSK2636771 discontinuation (except for 1 with normalized phosphate levels but persisting Grade 1 hypocalcemia); 2 of the 3 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; 60 patients experienced AEs that were considered to be related to the study drug (Table 2). The most common AEs across all dose levels (any; treatment-related) were gastrointestinal (diarrhea [n=31, 48%; n=27, 42%], nausea [n=27, 41%; n=18, 28%], vomiting [n=20, 31%; n=12, 18%]), and fatigue (n=17 [26%], n=14, 22%) (Table 2). AEs by dose-level are shown in Supplementary Table 3. Overall, 10 (15%) patients had treatment permanently discontinued due to an AE. Reasons for discontinuation were fatigue (n=1 in the 25 mg dose escalation cohort); fatigue/nausea (n=1) and hypophosphatemia/hypocalcemia (n=1), both in the 500 mg dose escalation cohort; proteinuria (n=1; 200 mg); pain (n=1), increased N-terminal prohormone brain natriuretic peptide/upper respiratory tract infection (n=1) and small intestinal obstruction (n=1), all with 350 mg of GSK2636771; and dyspnea (n=1), fatigue/vomiting (n=1), and urinary retention (n=1), all in the 400 mg expansion cohort. Dose reductions were required during trial treatment by 4 of 18 (22%) patients treated at the RP2D of 400 mg QD. Forty-one serious AEs (SAEs) occurred in 24 (37%) patients. Nine SAEs in 5 patients were considered related to the study drug (nausea, fatigue, hypocalcemia, and hypophosphatemia in 1 patient, increased creatinine and decreased appetite in 1 patient, and vomiting, urinary retention and pruritic rash in 1 patient each). Apart from the Grade 1 increased creatinine, related SAEs were Grade 2 or 3. One patient experienced dyspnea which had a fatal outcome, but the event was considered not related to study treatment. The primary cause of death for this patient was disease under study. 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 2 (3%) incidences of hyperglycemia were reported as AEs (Grade 1 in 1 patient and Grade 2 in the other). No cases of drug-related grade ≥3 hyperglycemia were observed in this study. Cutaneous toxicity was uncommon: 10 cases (15%) of skin rash were documented across all dose levels (4 of which were in the GSK2636771 400 mg QD group). In addition, 3 cases of pruritic rash (200 mg and 500 mg QD dose escalation cohorts and 400 mg QD expansion cohort) and 3 cases of maculopapular rash (50 mg QD PD, 350 mg QD dose escalation, and 400 mg QD expansion cohorts) were reported. Other than the aforementioned cases of hypophosphatemia and hypocalcemia, evidence for renal tubular toxicity also included proteinuria, which was reported in 4 (6%) patients: 1 in each of the GSK2636771 50 mg, 200 mg, 350 mg, and 500 mg QD dose groups.

PK-PD

PK-PD data are reported for the N=63 cohort. 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 half-life between 13 and 23 hours. Single-dose PK data are shown in Supplementary Tables 4 and 5. Similar dose-proportional findings (for both $AUC_{(0–\tau)}$ and C_{max}) were observed after repeated daily oral dosing at Day 22, with ratio of $AUC_{(0-\infty)}$ versus $AUC_{(0–\tau)}$ suggesting steady-state had been achieved (Table 3 and Supplementary Table 6). PK parameters for the PD cohorts and concentrations in patients (n=2) in Part 3 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 from preclinical experiments. 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 $AUC_{(0-\tau)}$ had a geometric mean of 205 μg/mL.

Inhibition of PI3K signaling was observed in 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 (data not shown).

In 4/5 (80%) patients who received the RP2D of 400 mg QD decreases in pSer473 AKT and its downstream target (pThr246 PRAS40) were observed in paired tumor biopsies (pre- and on-treatment) (Figures 2C and 2D). Decreases in pSer235/236 S6RP and pThr308 AKT were also observed in 2/4 (50%) and 2/5 (40%) of evaluable patients, respectively (Figures 2C and 2D).

Antitumor activity

Of the total 65 enrolled patients, we observed 1 partial response (PR), 21 stable disease (SD); 3 non-complete response (CR)/non-progressive disease (PD); 35 PD and 5 non-evaluable investigator-assessed best responses (based on RECIST 1.1 criteria). No CRs were reported. One patient with metastatic CRPC treated with GSK2636771 200 mg QD during the dose-escalation phase experienced a confirmed PR, as well as a 78% fall in his prostate-specific antigen levels. The response was durable with progression after 68 weeks of treatment (Figure 3A–C). An additional 10 patients treated with GSK2636771 remained on therapy and free of progression for at least 24 weeks, including 2 more patients with CRPC (34 and 57 weeks), 5 colorectal (25–33 weeks), 1 endometrial (33 weeks), 1 gastric (25 weeks) and 1 non-small cell lung cancer (33 weeks).

Association between antitumor activity and genomic biomarkers

Archival or fresh tumor biopsy samples from 55 patients participating in the study were retrieved. Of those, 48 (87%) passed quality control for next-generation sequencing. Overall,

5 patients had *PIK3CB* copy number gains or a putatively activating mutation (Figure 3D and Supplementary Tables 7–9); upon including the 2 additional patients with known *PIK3CB* aberrations at enrollment, 3/7 (43%) patients with *PIK3CB* genomic aberrations were on trial for ≥6 months. All 3 of these patients had CRPC, and remained on GSK2636771 treatment for 34, 57, and 68 weeks, respectively (the latter being the single patient with a *PIK3CB* copy number gain, exhibiting an investigator-assessed radiological PR). A cervical cancer patient harboring a known *PIK3CB* copy number gain received 22 weeks of GSK2636771 treatment, and showed a differential radiological response in lymph nodes (best overall response: SD).

Lastly, we analyzed the frequency of genomic events in other tumor suppressor and cancer promoting genes in the trial population. *PIK3CA* activating mutations were identified in 11 patients, with 1 additional patient showing a *PIK3CA* copy gain; 9 of these tumors harbored mutations in the RAS/RAF pathway, 3 in ataxia telangiectasia mutated (*ATM*), a key element of DNA damage response, and one in *BRAF* (Figure 3D). Interestingly, all but one of these were mutually exclusive with *PIK3CB* aberrations in this population and did not correlate with antitumor responses.

The p.L1049R mutation identified in a patient with CRPC, who achieved prolonged SD, is homologous to the very well-characterized *PIK3CA* hotspot mutation p.H1047R reported in cancer (Supplementary Figure 4A and 4B). 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 WT or the p.L1049R mutant at a range of multiplicity of infections. After 12 hours, substantially higher levels of pSer473 AKT were observed in cells with the p.L1049R mutation compared with WT cells (Supplementary Figure 4C), 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* (30,31).

Discussion

We here report on a first-time-in-human trial of GSK2636771, an oral selective PI3Kβ inhibitor. DLTs were identified and guided the selection of the RP2D, which was also supported by PK/PD data. Renal tubular damage, presenting in the form of hypophosphatemia, hypocalcemia, and proteinuria, was dose dependent, reversible, and manageable. Hyperglycemia and rash, typically reported for pan PI3K inhibitors, were uncommon. Also, GSK2636771 did not elevate insulin levels in mice compared with a pan PI3K/mTOR inhibitor. 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).

Target inhibition was demonstrated at tolerated doses. Repeat-dose exposure appeared to increase in a generally dose-proportional manner. GSK2636771 doses >200 mg QD consistently resulted in blood concentrations >0.6 μg/mL, the level predicted to robustly inhibit PI3Kβ from preclinical experiments. The observed inhibitory effect of GSK2636771 on pAKT (Ser473) and other biochemical markers (eg, pGSK3β [Ser9]) 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 its selection.

Several genomic landscape studies of different tumor types have identified that the *PI3K/AKT* pathway is altered in squamous cell lung (33), endometrial and head and neck cancers (34) and advanced prostate (35) and ovarian (36) cancers. However, in tumor types where activation of *PIK3CA* is more common, such as breast or colorectal cancer, genomic aberrations in *PIK3CB* are rare (<2%) (3,37). We, therefore, pursued retrospective tumor-targeted next-generation sequencing to explore putative predictive biomarkers of antitumor activity.

Activating mutations in *PIK3CA* have been previously associated to responses to pathway inhibitors (38); mutations leading to activation of *PIK3CB* have been reported in different tumour types but their clinical relevance remains to date unknown (31,39). Of 48 samples analyzed in the current study, interestingly, 5 (10%) had *PIK3CB* aberrations, namely 4 patients with copy number gains and 1 with an activating mutation (p.L1049R). Additionally, 2 patients harboring *PIK3CB* copy number gains that were previously determined were also enrolled in the expansion phase. Among these 7 patients, we observed one durable radiological PR (on treatment for 68 weeks) and prolonged SD (on treatment for 34 and 57 weeks) in the CRPC subset. This association, albeit preliminary, is of particular interest in advanced prostate cancer, where molecular stratification for therapy selection remains an unmet medical need. However, the patient population investigated here was very small and further studies are needed to fully determine the role of *PIK3CB* aberrations and indeed other biomarkers in the molecular stratification of patients when targeting this pathway. Importantly, several patients without *PIK3CB* aberrations benefited from therapy with prolonged SD. Therefore, *PIK3CB* aberrations do not fully explain the responses observed, highlighting the complexity of the PI3K/AKT/mTOR signaling pathway and the likely need for combination therapy to drive robust anti-tumor responses. The study was limited by the pre-selection of patients with PTEN-deficient tumors, which did not allow for assessment of the impact of genetic aberrations in non-PTEN deficient tumors. Moreover, mostly archival rather than fresh tumor biopsies were analyzed which precluded the detection of aberrations that may emerge during tumor evolution (35,40). Despite this, the preliminary results presented here are of interest and form the basis for further studies into the association of *PIK3CB* aberrations with clinical benefit. Further studies will also be needed to better characterize the mechanisms associated with tubular damage at high doses, although these were not a concern at the RP2D.

In conclusion, 400 mg QD continuous dosing was established as the RP2D for GSK2636771 based on DLTs. 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 CRPC, in combination with paclitaxel in gastric cancer, and in combination with immunotherapy in melanoma.

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Authors' contributions

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

Development of methodology: GG, JW, MM, RK, JSdB, KS, SD

Acquisition of data: JM, GG, HAB, MPD, DAS, SKS, MM, S-WH, S-YR, HCC, JPE, SS, Y-JB, JRI, JSdB, H-TA, JMT, SYR, KS, SD, CL

Analysis and interpretation of data: JM, GG, MPD, DAS, SK-S, JW, MM, RK, LY, JMT, JSdB

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

Administrative, technical, or material support: KS, SD

Study supervision: JSdB

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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)
Unknown	1 (2)
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)
ECOG status, n (%)	
0	37 (57)
1	28 (43)
Prior lines of therapy, n (%)	
1	7 (11)
2	9 (14)
3	4 (6)
4	8 (12)
>4	37 (57)

CNS, central nervous system; ECOG, Eastern Cooperative Oncology Group; GE, gastroesophageal junction; NSCLC, non-small cell lung cancer; SD, standard deviation.

Table 2. Summary of AEs and treatment-related AEs occurring in ≥10% of all patients, by grade

Preferred term, n (%)	Any AE		Treatment-related AE	
	Total (N=65)		Total (N=65)	
	Any grade	Grade ≥3	Any grade	Grade ≥3
Any AE	65 (100)	33 (51)	60 (92)	15 (23)
Hypophosphatemia	8 (12)	6 (9)	6 (9)	5 (7)
Rash	10 (15)	3 (5)	5 (8)	3 (5)
Fatigue	17 (26)	3 (5)	14 (22)	2 (3)
Hypocalcemia	9 (14)	2 (3)	6 (9)	2 (3)
Diarrhea	31 (48)	1 (2)	27 (42)	1 (2)
Nausea	27 (41)	2 (3)	18 (28)	1 (2)
Vomiting	20 (31)	2 (3)	12 (18)	1 (2)
Decreased appetite	15 (23)	1 (2)	9 (14)	1 (2)
Increased aspartate aminotransferase	8 (12)	2 (3)	5 (8)	1 (2)
Hypertension	6 (9)	2 (3)	3 (5)	1 (2)
Rash maculopapular	3 (5)	1 (2)	3 (5)	1 (2)
Rash pruritic	3 (5)	1 (2)	3 (5)	1 (2)
Hypoalbuminemia	4 (6)	2 (3)	2 (3)	1 (2)
Increased NT-proBNP	3 (5)	1 (2)	2 (3)	1 (2)
Proteinuria	4 (6)	1 (2)	2 (3)	1 (2)
Dehydration	3 (5)	3 (5)	1 (2)	1 (2)
Nephropathy	1 (2)	1 (2)	1 (2)	1 (2)
Urinary retention	2 (3)	1 (2)	1 (2)	1 (2)
Dysgeusia	4 (6)	0 (0)	4 (6)	0 (0)
Headache	13 (20)	1 (2)	4 (6)	0 (0)
Increased blood creatinine	9 (14)	0 (0)	4 (6)	0 (0)

AE, adverse event; NT-proBNP, N-terminal prohormone brain natriuretic peptide.

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

PK parameter, n (%)	Dose selection cohort	Dose escalation cohort						
	25 mg	**25 mg**	**50 mg**	**100 mg**	**200 mg**	**350 mg**	**400 mg**	**500 mg**
	n=3	**n=6**	**n=4**	**n=3**	**n=3**	**n=7**	**n=6**	**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	n=2	n=5	n=4	n=3	n=3	n=4	n=6	n=2
(range)	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)
$AUC_{(0–T)}$, 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%).

$AUC_{(0–T)}$, area under the time concentration-time curve over the dosing interval; C_{max}, maximum observed plasma concentration; CVb, coefficient of biological variation; PK, pharmacokinetic; 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 using 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 WT with PTEN-deficient cells. Error bars correspond to SD. **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 prostate cancer cells were treated with GSK2636771 1 or 10 μM 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 pAKT 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 SD.

AKT, protein kinase B; mTOR, mammalian target of rapamycin;
PD, pharmacodynamics; PI3K, phosphoinositide 3 kinase; PK, pharmacokinetic;
PTEN, phosphatase and tensin homolog, SD 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 (n=3 replicates per sample). **C,** changes in pSer473, pThr246 PRAS40, pSer235/236 and pThr308 were measured in tumor biopsies using IHC (H-scores) at pre-treatment and Days 8–15 (2–4h post dose) (n=1). **D,** representative photomicrographs (20x magnification) of IHC staining showing tumor PD effects of p-AKT (Ser473, Thr308), p-PRAS40 (Thr246) and pSer6RP (Ser235/236) for 2 patients treated with GSK2636771 400 mg QD (melanoma and TNBC). Biopsies were collected 2–4 hours post dose between Day 8 and 15. *p<0.05 Paired *t*-test.

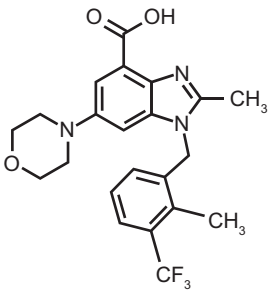
AKT, protein kinase B; PD, pharmacodynamics; QD, once daily; TNBC, triple negative brain cancer.

Figure 3. Summary of antitumor activity of GSK2636771 in the first-in-man trial, and association with putative predictive biomarkers.

A, Treatment duration in the 65 patients showed that 1 patient with CRPC had a PR, 24 patients had SD or non-CR/non-PD, 7 of whom received GSK2636771 treatment for ≥6 months. **B,** The PR 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 68 weeks of treatment (n=1). **D,** Summary of 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 (gray). CNV was determined based on Nanostring or qPCR platforms.

CNS, central nervous system; CNV, copy number variation; CRPC, castrate-resistant prostate cancer; GE, gastroesophageal junction; PD, pharmacodynamics; PR, partial response; PSA, prostate-specific antigen; qPCR, quantitative polymerase chain reaction.

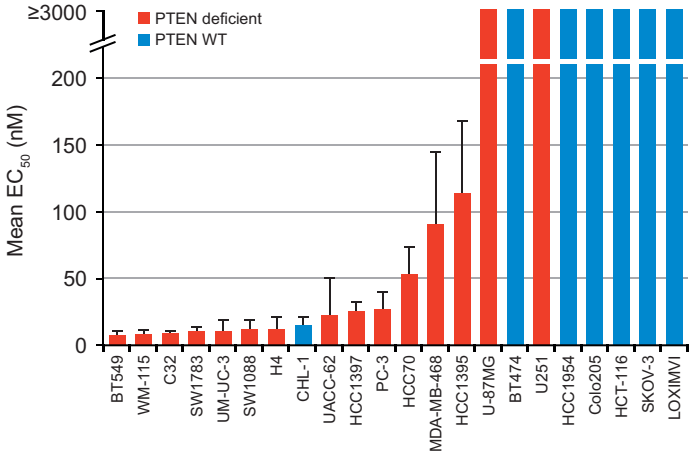
A



B

PI3K family	Human enzyme	Enzyme assay Mean IC_{50} (nM) ± SD	Kinobeads extract Mean kD (nM)
Class I	PI3Kβ	5.2 ± 2.9	1.2
	PI3Kα	>5,800	>30,000
	PI3Kδ	58 ± 32	234.4
	PI3Kγ	>12,600	6760.8
Class II	PIK3C2B	>20,000	>30,000
Class III	VPS34	6,310	8511.4
Class IV	DNA–PK	>50,000	>30,000
	mTOR	>50,000	>30,000

C



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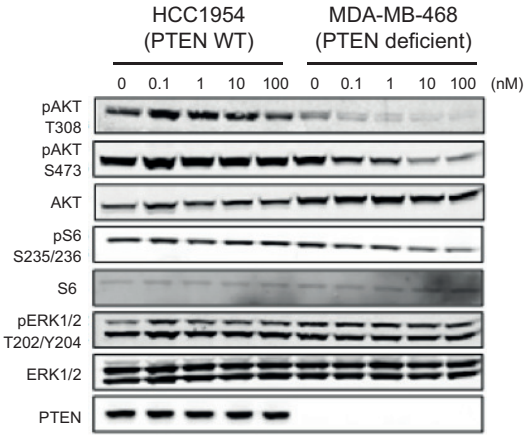

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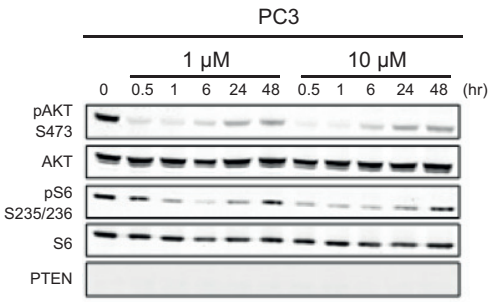

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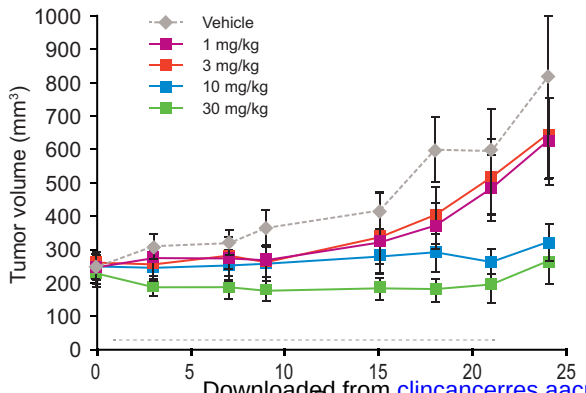

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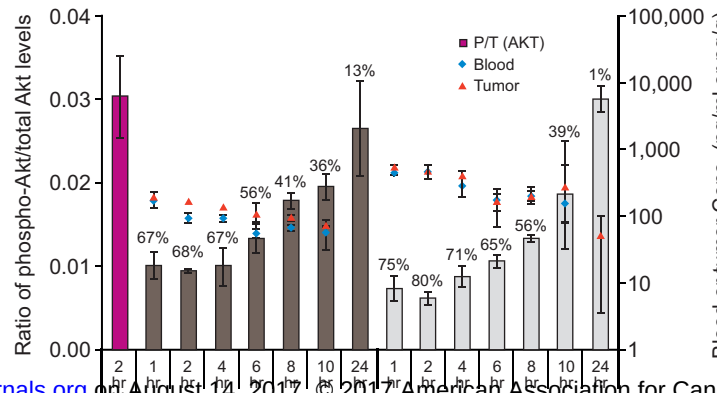

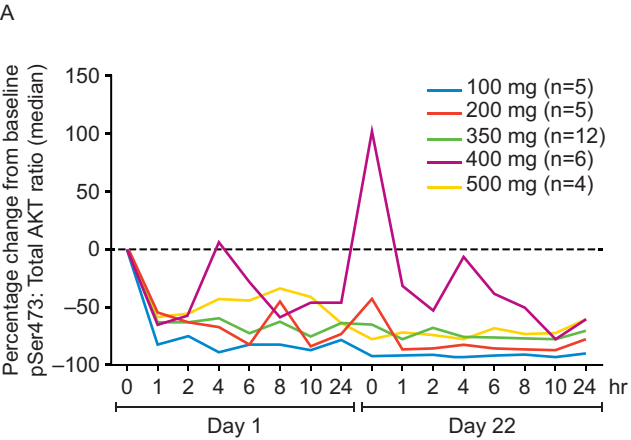

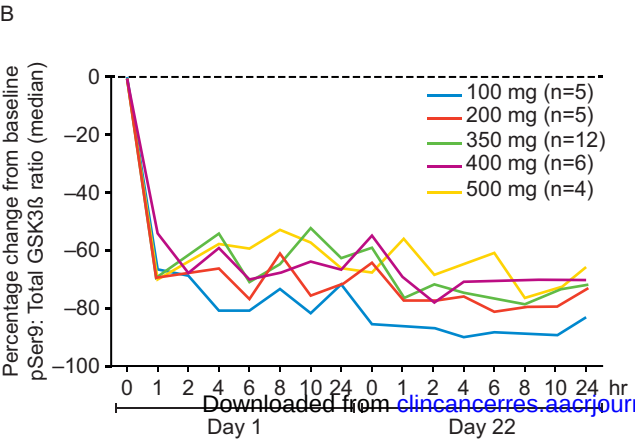

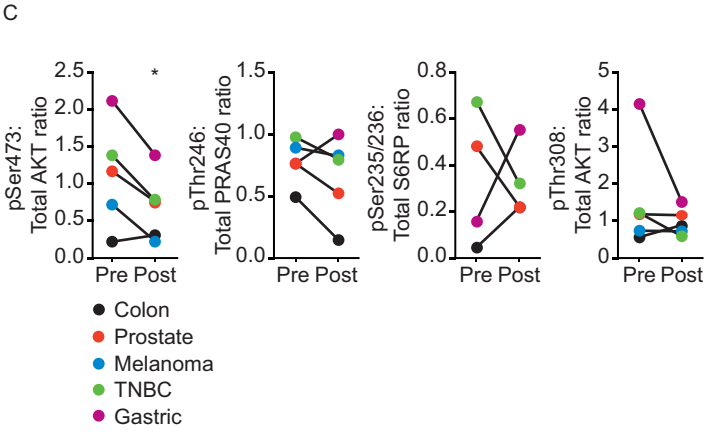

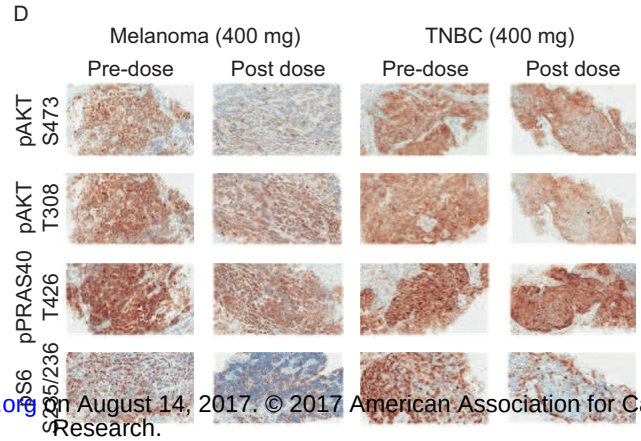

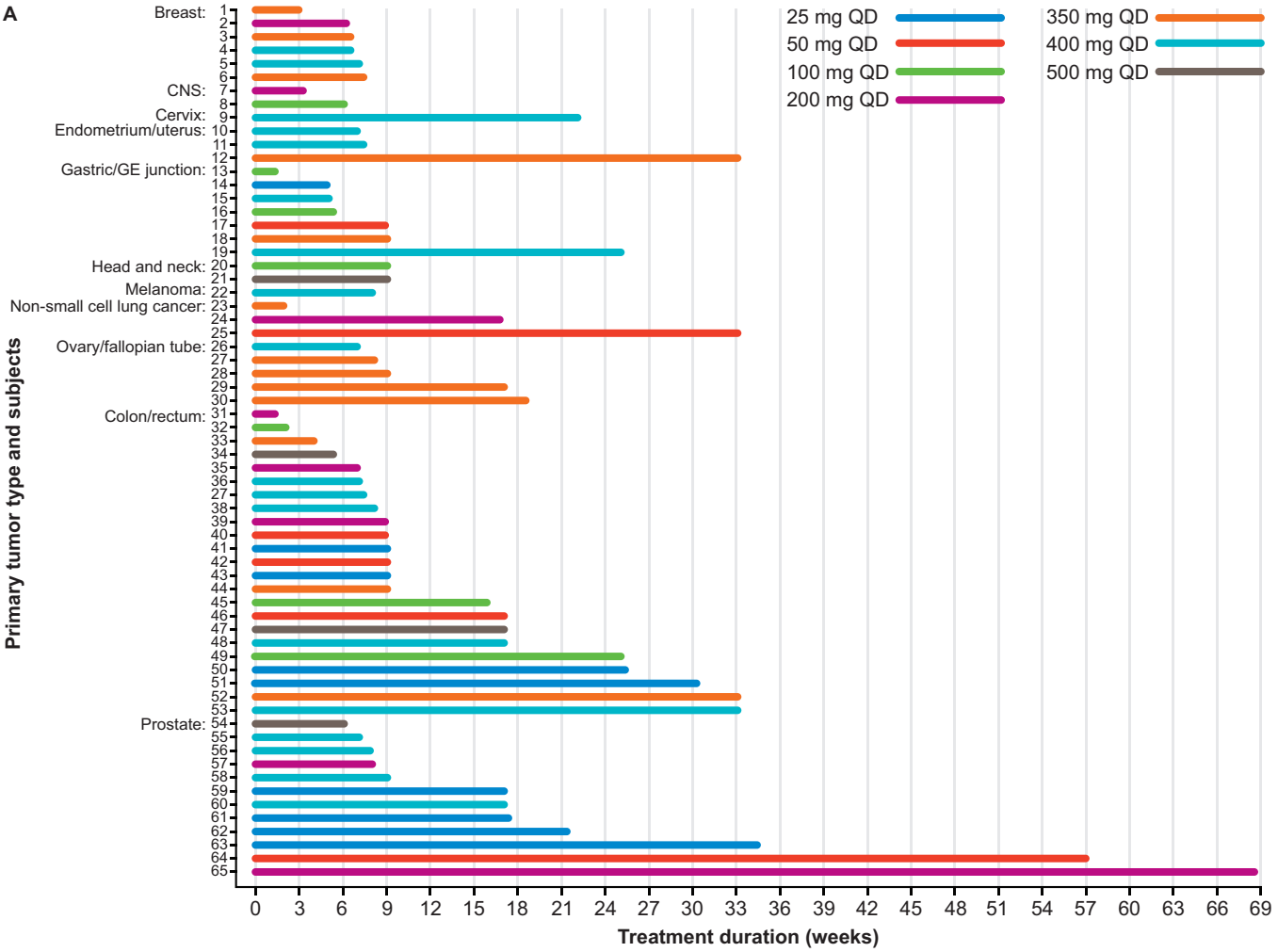

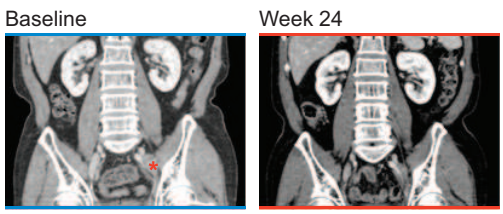

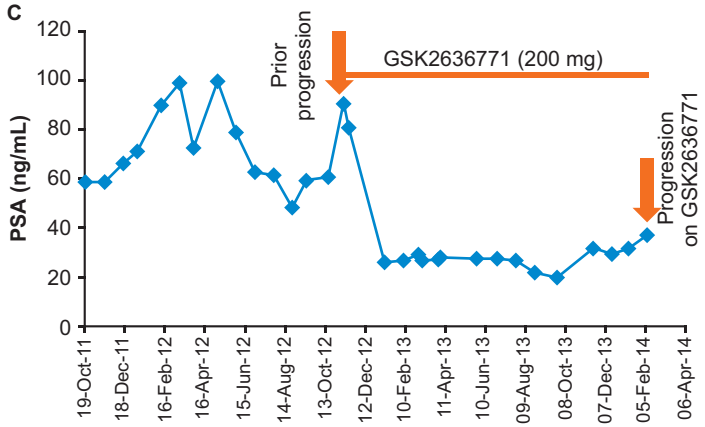

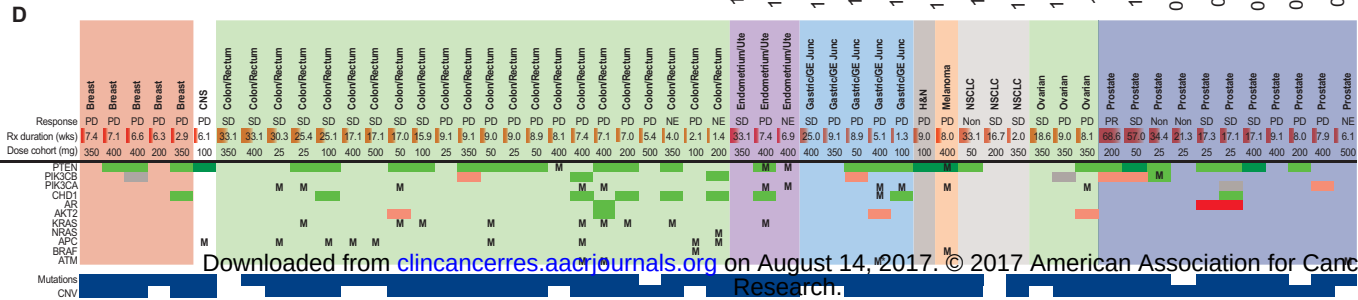

AACR American Association for Cancer Research

Clinical Cancer Research

A first-time-in-human study of GSK2636771, a phosphoinositide 3 kinase beta-selective inhibitor, in patients with advanced solid tumors

Joaquin Mateo, Gopinath Ganji, Charlotte Lemech, et al.

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