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Initial Testing (Stage 1) of the Akt Inhibitor GSK690693 by the Pediatric Preclinical Testing Program

Hernan Carol, PhD¹, Christopher L. Morton, BS², Richard Gorlick, MD³, E. Anders Kolb, MD⁴, Stephen T. Keir, PhD⁵, C. Patrick Reynolds, MD, PhD⁶, Min H. Kang, PharmD⁶, John M. Maris, MD⁷, Catherine Billups, MS², Malcolm A. Smith, MD, PhD⁸, Peter J. Houghton, PhD⁹, and Richard B. Lock, PhD¹

¹ Children's Cancer Institute Australia for Medical Research, Randwick, NSW, Australia

² St. Jude Children's Research Hospital, Memphis, TN

³ The Children's Hospital at Montefiore, Bronx, NY

⁴ A.I. duPont Hospital for Children, Wilmington, DE

⁵ Duke University Medical Center, Durham, NC

⁶ Texas Tech University Health Sciences Center, Lubbock, TX

⁷ Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine and Abramson Family Cancer Research Institute, Philadelphia, PA

⁸ Cancer Therapy Evaluation Program, NCI, Bethesda, MD

⁹ Nationwide Children's Hospital, Columbus, OH

Abstract

Background—GSK690693 is a small molecule ATP-competitive inhibitor of the pro-survival kinase Akt. Since Akt regulates multiple downstream targets including transcription factors, glycogen synthase 3, the pro-apoptotic protein Bad, as well as MDM2 and mTORC1, it was tested against the *in vitro* and *in vivo* panels of the Pediatric Preclinical Testing Program (PPTP).

Procedures—GSK690693 was tested *in vitro* at concentrations from 1 nM to 10 μM, and against the *in vivo* panel of xenografts at a dose of 30 mg/kg daily x 5 for 6 consecutive weeks. Three measures of *in vivo* antitumor activity were used: 1) an objective response measure modeled after the clinical setting; 2) a treated to control (T/C) tumor volume measure; and 3) a time to event measure based on the median event-free survival (EFS) of treated and control animals for each xenograft.

Results—GSK690693 inhibited cell growth *in vitro* with IC₅₀ values between 6.5 nM and >10 μM. *In vivo*, GSK690693 significantly increased EFS in 11 of 34 (32%) solid tumor xenografts, most notably in all 6 osteosarcoma models, but not in any of the 8 ALL xenografts tested. No objective responses were observed and only one solid tumor met EFS T/C criteria for intermediate activity.

Conclusions—GSK690693 demonstrated broad activity *in vitro*, however our results against both the solid tumor and ALL PPTP *in vivo* panels demonstrate that, as single agent at the dose and schedule used, GSK690693 has only modest antitumor activity.

Corresponding Author: Richard B. Lock, PhD, Children's Cancer Institute Australia for Medical Research, Randwick, NSW, Australia, Voice: + 61 2 9385 2513, Fax: + 61 2 9662 6583, rlock@ccia.unsw.edu.au.

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Keywords

Preclinical Testing; Developmental Therapeutics; Akt inhibitor

INTRODUCTION

Akt kinases (or protein kinase B, PKB) are three serine-threonine kinase isoforms at the center of a complex pathway that senses growth factor stimulation and mediates cell proliferation, metabolism and death (reviewed in [1,2]). Constitutive Akt activation has been reported in a number of cancer types, and its activity has been linked to both oncogenesis and poorer prognosis [3,4]. Akt can be constitutively activated by mutation, or more commonly as a consequence of abnormal upstream signaling by modulators including phosphatidylinositol-3-kinase (PI3K), or by loss or mutations in negative regulators such as the serine phosphatase PTEN [5]. Also, constitutively active Akt has been shown to reduce cell sensitivity to pro-apoptotic drugs [6,7]. All of these factors have pointed to Akt as a very attractive target for pharmacological intervention in cancer [8,9].

Two phosphorylation sites regulate the activity of Akt; phosphorylation at threonine residue 308 (pAktThr308) is a requirement while further phosphorylation at serine 473 (pAktSer473) greatly increases enzymatic activity [10]. These events are mediated by the kinase PDK1 after Akt is recruited to the plasma membrane, and depend on levels of PI3, which are in turn controlled by PTEN [11]. Akt kinase activity regulates multiple downstream targets including FOXO transcription factors, glycogen synthase 3 isoforms, the pro-apoptotic protein Bad, and mTORC1, as well as apoptosis inhibitors such as mdm2 and XIAP [4].

The PI3K/Akt signaling pathway plays an important role in several cancer types, including pediatric cancers [4]. Akt phosphorylation is detectable in a number of pediatric solid tumors, including neuroblastoma [12], rhabdomyosarcoma [13], and Ewing sarcoma [14]. In neuroblastoma clinical specimens, AKT was highly phosphorylated at Ser473 in 61.2% (71 of 116) and at Thr308 in 62.9% (73 of 116) of cases, with 66 (56.9%) cases positive at both sites [12]. Akt activation is a marker of poor prognosis in neuroblastoma [12], in which both IGF-1R signaling [15,16] and BDNF/TrkB signaling [17–19] have been suggested as initiators of activation of PI3K and Akt. For rhabdomyosarcoma clinical specimens, pAktThr308 was elevated in 42% and 35% of alveolar and embryonal rhabdomyosarcoma cases, respectively, while pAktSer473 was increased in 43% of alveolar rhabdomyosarcoma and 55% of embryonal rhabdomyosarcoma. The latter phosphorylation was associated with lower overall survival ($p < 0.001$) and recurrence-free survival ($p < 0.0009$) [13]. Similarly, Akt activation has been demonstrated in several hematological malignancies [20].

GSK690693 is a small molecule inhibitor of the three isoforms of Akt, acting through ATP competition, with specificity limited to Akt (and to some extent the AGC kinase family) [21]. Its activity against different ALL cell lines and cancer models has been described elsewhere [22,23]. Currently a Phase I dose escalation clinical trial is underway for solid tumors and lymphoma patients [24]. GSK690693 was selected for systematic testing by the PPTP based on the relevance of both the target molecule and the PI3K/Akt pathway for cell survival and proliferation. This report describes testing of GSK690693 against the PPTP's *in vitro* panel of cell lines and *in vivo* xenograft solid tumor and ALL panels.

MATERIALS AND METHODS

In Vitro Testing

In vitro testing was performed using DIMSCAN, a semiautomatic fluorescence-based digital image microscopy system that quantifies viable (using fluorescein diacetate [FDA]) cell numbers in tissue culture multiwell plates [25]. Cells were incubated in the presence of GSK690693 for 96 hours at concentrations from 1 nM to 10 μ M and analyzed as previously described [26].

In Vivo Tumor Growth Inhibition Studies

CB17SC-M *scid*^{-/-} female mice (Taconic Farms, Germantown NY), were used to propagate subcutaneously implanted kidney/rhabdoid tumors, sarcomas (Ewing, osteosarcoma, rhabdomyosarcoma), neuroblastoma, and non-glioblastoma brain tumors, while BALB/c nu/nu mice were used for glioma models, as previously described [26–29]. Human leukemia cells were propagated by intravenous inoculation in female non-obese diabetic (NOD)/*scid*^{-/-} mice as described previously [30]. Female mice were used irrespective of the patient gender from which the original tumor was derived. All mice were maintained under barrier conditions and experiments were conducted using protocols and conditions approved by the institutional animal care and use committee of the appropriate consortium member. Ten mice (solid tumors) or eight mice (leukemias) were used in each control or treatment group. Tumor volumes (cm³) [solid tumor xenografts] or percentages of human CD45-positive [hCD45] cells [ALL xenografts] were determined as previously described [26] and responses were determined using three activity measures as previously described [26].

Determination of response—Responses were determined using three activity measures as previously described [26]. For individual mice, progressive disease (PD) was defined as < 50% regression from initial volume during the study period and > 25% increase in initial volume at the end of study period. Stable disease (SD) was defined as < 50% regression from initial volume during the study period and \leq 25% increase in initial volume at the end of the study. Partial response (PR) was defined as a tumor volume regression \geq 50% for at least one time point but with measurable tumor (\geq 0.10 cm³). Complete response (CR) was defined as a disappearance of measurable tumor mass (< 0.10 cm³) for at least one time point. A complete response was considered maintained (MCR) if the tumor volume was <0.10 cm³ at the end of the study period. For treatment groups only, if the tumor response was PD, then PD was further classified into PD1 or PD2 based on the tumor growth delay (TGD) value. TGD values were calculated based on the numbers of days to event. For each individual mouse that had PD and had an event in the treatment groups, a TGD value was calculated by dividing the time to event for that mouse by the median time to event in the respective control group. Median times to event were estimated based on the Kaplan-Meier event-free survival distribution. If a mouse had a TGD value \leq 1.5, that mouse was considered PD1. If the TGD value was > 1.5, the mouse was considered PD2. Mice that had PD but did not have an event at the end of the study were coded as PD2.

Event-free survival—An event in the solid tumor xenograft models was defined as a quadrupling of tumor volume from the initial tumor volume. Event-free survival was defined as the time interval from initiation of study to the first event or to the end of the study period for tumors that did not quadruple in volume. The time to event was determined using interpolation based on the formula:

$$t_x = t_1 + (t_2 - t_1) \ln(V_e/V_1) / \ln(V_2/V_1),$$

where t_x is the interpolated day to event, t_1 is the lower observation day bracketing the event, t_2 is the upper observation day bracketing the event, V_1 is the tumor volume on day t_1 , V_2 is the tumor volume on day t_2 and V_e is the event threshold (4 times initial tumor volume for solid tumor xenografts).

Response and Event Definitions for Acute Lymphoblastic Leukemia (ALL) Xenograft Models

Individual mice were categorized as PD if their percentage of hCD45 cells never dropped below 1% and they had an event before the end of the study period. An event is defined as hCD45 cells above 25% in the peripheral blood with times to event calculated as above. Individual mice were classified as SD if their percentage of hCD45 cells never dropped below 1% and no event occurred before the end of the study. PR was assigned if the percentage of cells dropped below 1% for any one time point regardless of whether the percentage eventually reached 25%. A CR was assigned if the percentage of hCD45 cells dropped below 1% for 2 consecutive weeks of the study and regardless of whether the percentage reached 25% or not. A CR was considered maintained if the percentage of hCD45 was less than 1% for the last three measurements of the study. For treatment groups, PD was further classified into PD1 and PD2 according to the TGD value.

The time to event was determined using interpolation based on the formula:

$$t_x = t_1 + (t_2 - t_1) \ln(V_e/V_1) / \ln(V_2/V_1),$$

where t_x is the interpolated day to event, t_1 is the lower observation day bracketing the event, t_2 is the upper observation day bracketing the event, V_1 is the hCD45 percentage on day t_1 , V_2 is the tumor volume (or hCD45 percentage) on day t_2 and V_e is the event threshold (25% for ALL xenografts).

Summary statistics and analysis methods

Overall Group Response—Each individual mouse was assigned a score from 0 to 10 based on their response: PD1=0, PD2=2, SD=4, PR=6, CR=8, and MCR=10, and the median for the group determined the overall response. Studies in which toxicity was greater than 25% or in which the control group was not at least SD, were considered inevaluable and were excluded from analysis. Treatment groups with PR, CR, or MCR are considered to have had an objective response. Agents inducing objective responses are considered highly active against the tested line, while agents inducing stable disease or PD2 are considered to have intermediate activity, and agents producing PD1 are considered to have a low level of activity against the tested line.

Tumor Volume T/C value—Relative tumor volumes (RTV) for control (C) and treatment (T) mice were calculated at day 21 or when all mice in the control and treated groups still had measurable tumor volumes (if less than 21 days). The mean relative tumor volumes for control and treatment mice for each study were then calculated and the T/C value was the mean RTV for the treatment group divided by the mean RTV for the control group. For the tumor volume T/C response measure, agents producing a T/C of $\leq 15\%$ are considered highly active, those with a mean tumor volume T/C of $\leq 45\%$ but $> 15\%$ are considered to have intermediate activity, and those with mean T/C values $> 45\%$ are considered to have low levels of activity [31].

EFS T/C value—An EFS T/C value was defined by the ratio of the median time to event of the treatment group and the median time to event of the respective control group. If the

treatment group did not have a median time to event, then EFS T/C was defined as greater than the ratio of the last day of the study for the treatment group divided by the median time to event for the control group. For the EFS T/C measure, agents are considered highly active if they meet three criteria: a) an EFS T/C > 2; b) a significant difference in EFS distributions ($p \leq 0.050$), and c) a net reduction in median tumor volume for animals in the treated group at the end of treatment as compared to at treatment initiation. Agents meeting the first two criteria, but not having a net reduction in median tumor volume for treated animals at the end of the study are considered to have intermediate activity. Agents with an EFS T/C < 2 are considered to have low levels of activity. Xenografts in which the median EFS for the control line was greater than one-half of the study period or in which the median EFS for the control line did not exist are considered not evaluable for the EFS T/C measure of activity.

Statistical Methods

The exact log-rank test, as implemented using Proc StatXact for SASR, was used to compare event-free survival distributions between treatment and control groups. P-values were two-sided and were not adjusted for multiple comparisons given the exploratory nature of the studies.

Drugs and Formulation

GSK690693 was provided to the PPTP by Glaxo Smith Kline, through the Cancer Therapy Evaluation Program (NCI). GSK690693 was dissolved in 5% (w/v) mannitol in saline and administered once daily via intraperitoneal (IP) injection using a 5 days on 2 days off for 6 weeks schedule at 30 mg/kg. For secondary screening in ALL, an alternative formulation [5% (w/v) mannitol in acetate buffer] was used, and the drug was administered twice daily. GSK690693 was provided to each consortium investigator in coded vials for blinded testing.

Pharmacodynamic Studies

Blood glucose was determined over time using an AccuCheck glucometer (Roche). Phosphorylation of Akt, S6 and total proteins was determined by western blot analysis as previously described [13,32]. Primary antibodies used were against α -tubulin (Santa Cruz Biotechnology), ribosomal protein S6 (rpS6), phospho-rpS6 (Ser235/236), Akt, and phospho-Akt(Ser473) (Cell Signaling Technologies). Immunoreactive bands were visualized using SuperSignal Chemiluminescence substrate (Pierce) and Biomax MR and XAR film (Eastman Kodak Co.).

RESULTS

GSK690693 *in Vitro* Testing

GSK690693 demonstrated highly variable activity against the PPTP's *in vitro* cell line panel. At one extreme were 5 cell lines having IC_{50} values greater than 10 μ M (Table I), while at the other extreme were five cell lines with IC_{50} values of approximately 100 nM or lower and showing a high degree of cytotoxic effect as evidenced by an estimated 80% or greater decrease in viable cells from input after 96 hours exposure (Table I). The median IC_{50} for all PPTP cell lines was 1.2 μ M, with the most sensitive being the T-cell ALL cell line COG-LL-317, which had an IC_{50} of 6.5 nM. The ALL panel showed the highest level of sensitivity (Figure 1A) with additional highly responsive cell lines including Kasumi-1 (an AML cell line with an ETO-AML1 translocation that is known to have a KIT activating point mutation [12]). For solid tumor cell lines the rhabdomyosarcoma cell line Rh41 was highly sensitive, (Figure 1B), whereas each of the neuroblastoma cell lines had an IC_{50} greater than 5 μ M with three showing IC_{50} values exceeding 10 μ M (Figure 1A). The profile of *in vitro* drug sensitivity of the resistant cell line NB-1643 is shown in Figure 1C.

Activity of GSK690693 Against the PPTP *In Vivo* Panel

Forty-two xenograft models were considered evaluable, with five xenografts excluded from reporting because of excessive toxicity. These five xenografts were from a cross section of panels, including the Wilms (KT-11), rhabdomyosarcoma, (Rh18), ependymoma (BT-36), and neuroblastoma (NB-SD and NB-1643) panels. Forty-six of 895 mice died during the study (5.1%), with 3 of 445 in the control arms (0.7%) and 43 of 450 in the GSK690693 treatment arms (9.6%). Complete details of testing are provided in Supplemental Table I, including total numbers of mice, number of mice that died (or were otherwise excluded), numbers of mice with events and average times to event, tumor growth delay, as well as numbers of responses and T/C values.

No objective responses were observed in any of the solid tumor panels, although GSK690693 induced significant differences in EFS distribution compared to controls in 12 of 34 evaluable solid tumor xenografts tested as shown in Table II. Significant differences in EFS distribution were observed for all 6 of the xenografts in the osteosarcoma panel and in one-half (2 of 4) of the xenografts from the glioblastoma panel. Significant differences in EFS distribution were also observed for xenografts in the rhabdomyosarcoma (1 of 6), neuroblastoma (1 of 4), rhabdoid (1 of 3) and ependymoma (1 of 1) panels. By contrast, none of the 8 evaluable ALL xenografts demonstrated significant differences in EFS distribution. Although there were significant differences in EFS distribution for selected solid tumor xenografts, the EFS T/C values were below the criteria for intermediate activity for the time to event measure of activity (EFS T/C > 2) in all but two evaluable lines: the rhabdoid tumor xenograft KT-12 and the osteosarcoma xenograft OS-33. Growth curves for these xenografts are shown in supplementary material (Figure 2).

For the tumor volume T/C activity measure, significant differences in tumor volume were observed for 11 xenografts, including all six xenografts in the osteosarcoma panel (Table II). Only one xenograft showed intermediate activity for this measure, the osteosarcoma xenograft OS-33 (T/C = 0.21). Of note, OS-33 was the only PPTP xenograft that also had a maintained complete response to rapamycin [33].

No objective responses were observed in any of the ALL models (Supplemental Figure 1). The best response was PD2 (progressive disease with growth delay), which was observed for two xenografts in the ALL panel, and eight xenografts in the solid tumor panel. The objective response measure results for both solid tumors and leukemia models are depicted in a 'COMPARE' format, based on the objective response scoring criteria centered around the midpoint score of 5 that represents stable disease (Figure 3).

Secondary Testing of ALL Models

Taking into consideration the lack of efficacy of GSK690693 against the ALL xenograft panel reported above, an additional *in vivo* efficacy experiment was conducted using a different formulation of GSK690693 against ALL-19, since this xenograft exhibited the greatest degree of sensitivity of the entire ALL panel to the drug *in vitro* (data not shown). GSK690693 was prepared in 5% (w/v) mannitol-acetate buffer and administered by IP injection on a twice-daily schedule at 30 mg/kg. Mice engrafted with ALL-19 and treated with the new formulation of GSK690693 exhibited no leukemia delay and did not survive longer than untreated control mice (median survival of control mice 6.5 days compared with 5.5 days for GSK690693-treated mice, $P=0.88$). The median objective response measure for ALL-19 engrafted mice treated with the new formulation of GSK690693 was classified as progressive disease, which is identical to that obtained with the original formulation and administration schedule.

Pharmacodynamic Studies

The systemic on-target effects of GSK690693 following IP injection were assessed by blood glucose monitoring. GSK690693 was prepared in both the mannitol-saline and mannitol-acetate (containing no sodium chloride) solutions described above. Groups of three non-engrafted NOD/SCID mice were given IP injections of vehicle control, mannitol-saline or mannitol acetate formulations of GSK690693 and blood glucose levels were assessed over time. Blood glucose levels are reported to be at a basal level of 5.6 – 8.9 mM. Both formulations of GSK690693 increased blood glucose levels relative to the vehicle control (Figure 4). The mannitol-saline formulation induced a blood glucose peak of ~18 mM at 2 h after administration, which then decreased, whereas the mannitol-acetate formulation induced a comparable blood glucose peak of ~19 mM at 4 hours after administration.

Phosphorylation of Akt (Ser473) and phospho-S6 protein (Ser 235/6) was also examined in five osteosarcoma xenografts from mice treated with GSK690693. GSK690693 markedly increased the levels of Ser473 phosphorylation between 1 and 8 hours post administration of drug in all xenografts (Figure 5). However, by 24 hours the level of phospho-Akt had returned to baseline. In contrast there was relatively little effect of drug administration on S6 phosphorylation.

DISCUSSION

The results obtained *in vitro* with the cell line panel show heterogeneity of sensitivity, although IC₅₀ values for only five of the cell lines were in the 100 nM range (or lower). There was a trend towards more effective cell kill in the ALL panel and more resistance among the neuroblastoma cell lines. These results are in contrast with those obtained *in vivo*, where only two xenograft lines achieved an intermediate level of activity and no objective responses were obtained. Further, two ALL xenografts showed very limited sensitivity to GSK690693, when tested *in vitro* with IC₅₀ values >10 μM (data not shown). It is possible, therefore, that the cell lines tested *in vitro* had become growth factor independent (for instance PTEN deficient) and as a consequence dependent to the PI3K-PTEN-Akt pathway, and more sensitive to its inhibition. Alternatively, we speculate that compensatory kinase activity present in the *in vivo* setting, but not *in vitro*, could explain these results. The GSK690693 results are consistent with the observation that pharmacological targeting of single kinases frequently results in limited efficacy (reviewed in [34]), although this does not imply that the *in vivo* therapeutic window will be increased by simultaneously targeting multiple signaling pathways.

One implication of the pattern of induction of pAktSer473 observed following treatment with GSK690693 is that it may reflect a homeostatic mechanism that could reduce the antitumor effect of GSK690693 when used as a single agent. A second observation is that the time course of Akt phosphorylation (and downstream blood glucose levels) with the return to baseline by 24 hours suggests that an alternative dosing schedule may show greater activity as a single agent. However, when we tested the efficacy of this drug on a twice daily schedule against the xenograft ALL-19 no significant improvement in efficacy was achieved.

There are some similarities between the *in vitro* and *in vivo* responses of the PPTP's preclinical models to GSK690693 and to rapamycin. Within several tumor panels, xenografts that showed the greatest response to rapamycin also tended to show the greatest response to GSK690693. For example, the OS-33 xenograft, which had the clearest *in vivo* response to GSK690693, was one of the most responsive xenografts to rapamycin [33]. However, the *in vivo* responses to rapamycin exceeded those observed for GSK690693, both for the solid tumor panels and for the ALL panel [33].

Rapid induction of pAktSer473 has been described following exposure to small molecule AKT inhibitors [35,36]. This induction of pAktSer473 occurs within minutes of exposure and is more rapid than the induction of phosphorylation that occurs following mTOR inhibition [35]. Phosphorylation at Ser473 requires continued PI3K and mTORC2 activity, but unlike rapalog-induced pAktSer473 does not appear to involve the mTORC1-S6K1 pathway that regulates IRS-1 stability [35]. Further evaluation is required to define the precise mechanism for this extremely rapid induction of pAktSer473 following AKT inhibition. However, because of the known activation of AKT as a consequence of mTOR inhibition, combining rapamycin with GSK690693 treatment could result in significant synergy. For example, combination of an Akt inhibitor with rapamycin completely suppressed hypoxia-induced increases in VEGF whereas single agents were less effective [32].

Given the activities observed *in vitro* for GSK690693 against ALL cell lines and those previously reported [22], the efficacy of the agent *in vivo* against the ALL xenografts is disappointing. There is no obvious explanation for the discrepant results between *in vitro* and *in vivo* testing for the ALL panels but these results reinforce the need for caution when extrapolating activities from *in vitro* testing on cell lines. Akt has been reported as a particularly relevant target in T-ALL [5,37] but we did not observe significant efficacy against the two T-lineage xenografts tested *in vivo*. The lack of activity observed for the ALL xenografts *in vivo* points to the difficult challenges that lay ahead of drug development efforts directed towards drugable kinase targets. However, whether the limited activity of this Akt inhibitor is intrinsic to this molecule, or representative of other inhibitors of this target remains to be tested.

In summary, the Akt inhibitor GSK690693 showed limited activity against the PPTP's *in vivo* xenograft panels. For the solid tumor panels, the most consistent antitumor effects were noted for the osteosarcoma panel, although the magnitude of these effects was relatively small. The xenograft that showed the greatest response to GSK690693 (OS-33) had stable disease for several weeks prior to progression. This xenograft is the only osteosarcoma xenograft of the PPTP panel that has very low IGF-1R expression, and achieved a complete response to rapamycin as single agent [33]. Overall, these results indicate that the use of GSK690693 as a single agent in the treatment of pediatric malignancies is likely to be of limited value without additional optimization.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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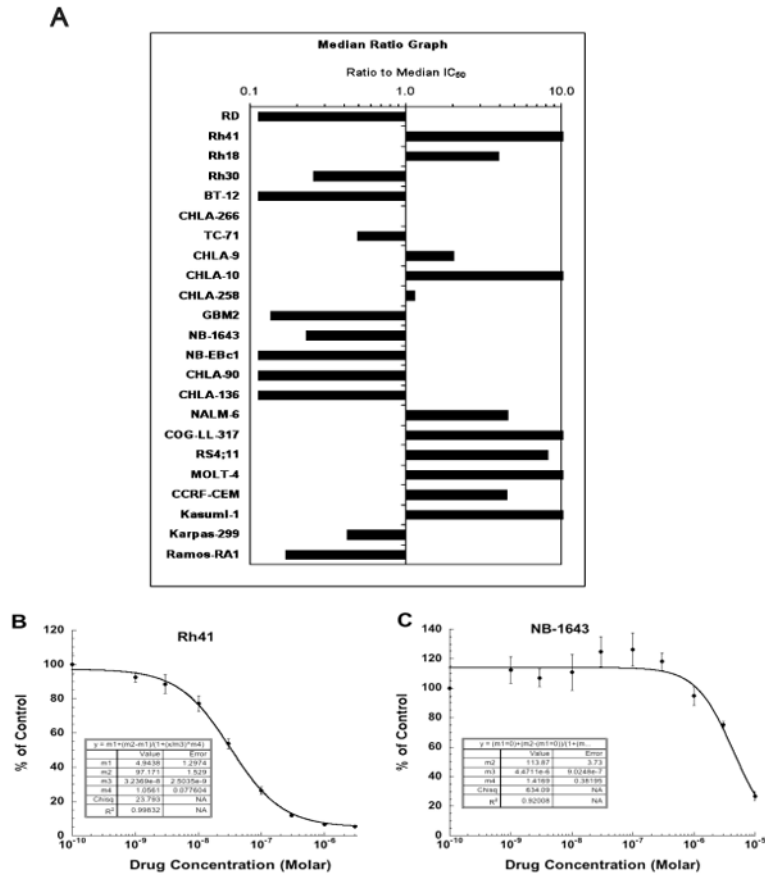


Figure 1. GSK690693 *in vitro* activity: (A) The median IC₅₀ ratio graph shows the relative IC₅₀ values for the cell lines of the PPTP panel. Each bar represents the ratio of the panel IC₅₀ to the IC₅₀ value of the indicated cell line. Bars to the right represent cell lines with higher sensitivity, while bars to the left indicate cell lines with lesser sensitivity. Representative dose response curves for Rh41 (B) and NB-1643 (C) cell lines.

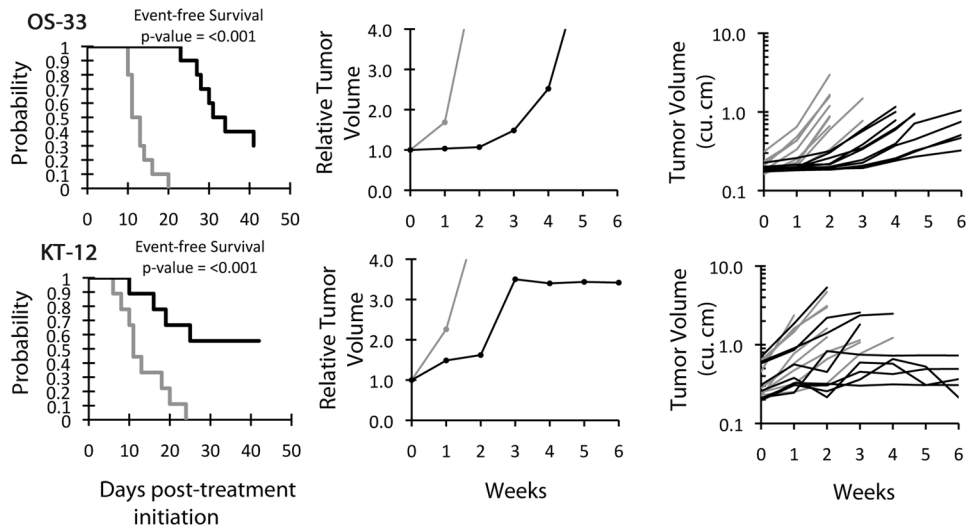


Figure 2. Plots depicting tumor growth and survival of mice treated with GSK960693 versus controls. Median tumor volume over time (left) and Kaplan-Meier plot of EFS over time (right) for the xenografts OS-33 (A) and KT-12 (B). Gray lines indicate vehicle-treated control mice and black lines indicate GSK690693-treated mice.

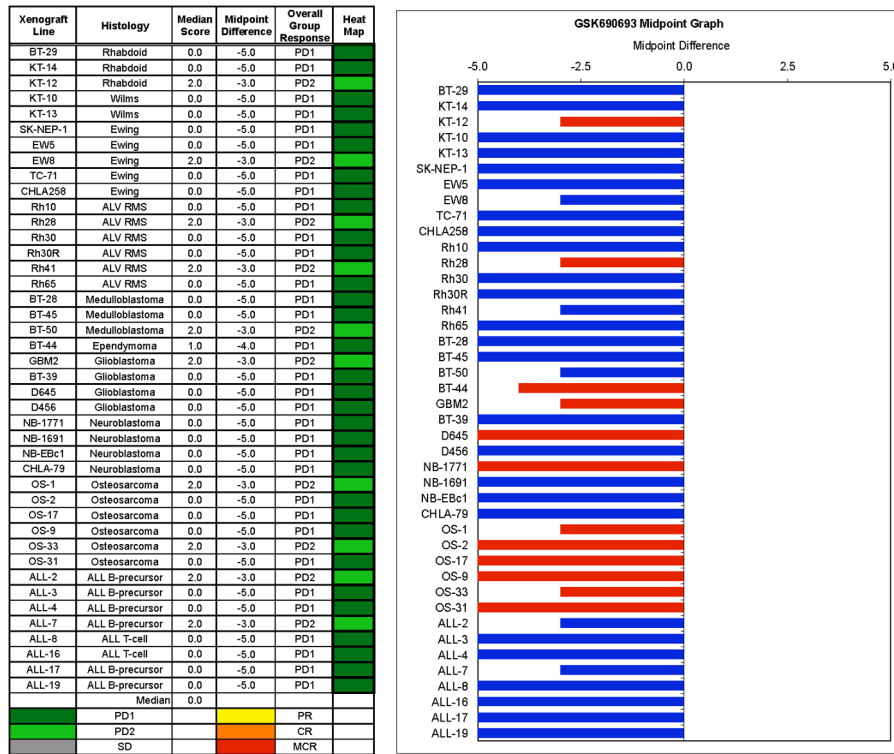


Figure 3. GSK690693 *in vivo* objective response activity: **Left:** The colored heat map depicts group response scores. A high level of activity is indicated by a score of 6 or more, intermediate activity by a score of ≥ 2 but < 6 , and low activity by a score of < 2 . **Right:** Representation of tumor sensitivity based on the difference of individual tumor lines from the midpoint response (stable disease). Bars to the right of the median represent lines that are more sensitive, and to the left are tumor models that are less sensitive. Red bars indicate lines with a significant difference in EFS distribution between treatment and control groups, while blue bars indicate lines for which the EFS distributions were not significantly different.

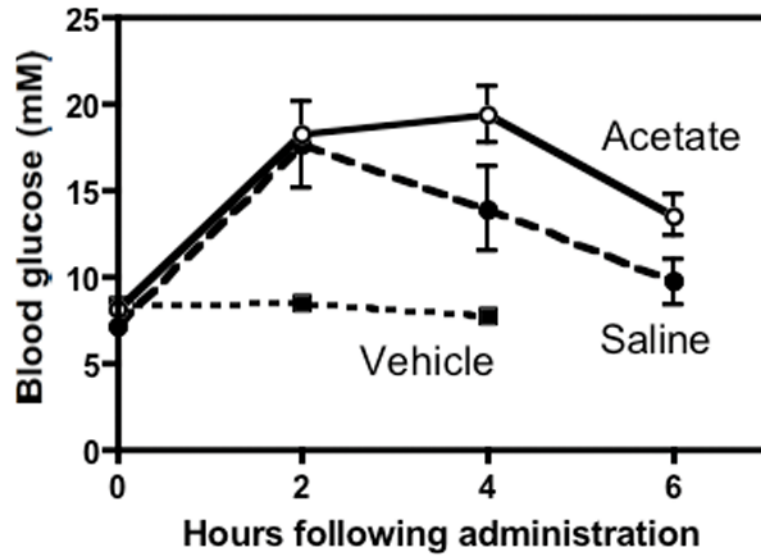


Figure 4. Blood glucose levels of NOD/SCID mice over time (n=3 per group) after a single injection of vehicle (back dashed line), 30 mg/kg GSK690693 in mannitol-saline (red line) or 30 mg/kg GSK690693 in mannitol-acetate (blue line).

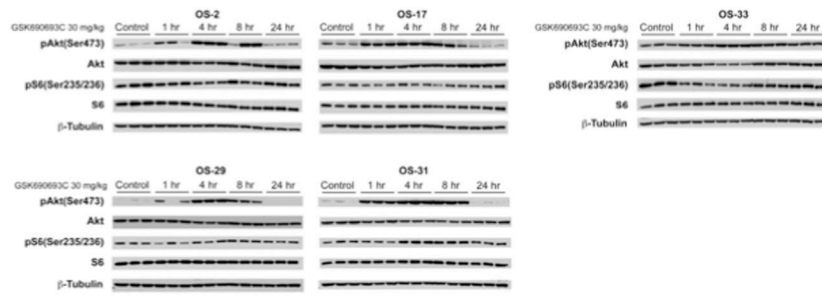


Figure 5. Pharmacodynamic evaluation of Akt inhibition. Western blot analyses were performed as previously described with minor modifications [13]. Each lane represents an individual sample of the osteosarcoma xenograft identified above the lanes collected either prior to or at the specified times after treatment with GSK690693. β -tubulin was used as a loading control.

Table 1

Activity of GSK690693 Against the PPTP *in Vitro* Panel

CELL LINE	Histology	(T/C) _{Min} %*	Relative I/O**	IC50	Median IC ₅₀ Ratio
RD	Rhabdomyosarcoma	61.2	0.57	>1 x 10 ⁻⁵	0.1
Rb41	Rhabdomyosarcoma	5.2	-0.88	3.38 x 10 ⁻⁸	36.1
Rb18	Rhabdomyosarcoma	36.4	-0.37	3.03 x 10 ⁻⁷	4.0
Rb30	Rhabdomyosarcoma	39.7	0.26	4.81 x 10 ⁻⁶	0.3
BT-12	Rhabdoid	71.5	0.65	>1 x 10 ⁻⁵	0.1
CHLA-266	Rhabdoid	32.9	-0.22	1.22 x 10 ⁻⁶	1.0
TC-71	Ewing sarcoma	16.5	0.13	2.52 x 10 ⁻⁶	0.5
CHLA-9	Ewing sarcoma	27.3	0.00	5.91 x 10 ⁻⁷	2.1
CHLA-10	Ewing sarcoma	16.3	0.05	1.02 x 10 ⁻⁷	12.0
CHLA-258	Ewing sarcoma	24.1	-0.49	1.05 x 10 ⁻⁶	1.2
GBM2	Ewing sarcoma	45.7	0.28	9.15 x 10 ⁻⁶	0.1
NB-1643	Neuroblastoma	26.8	0.19	5.40 x 10 ⁻⁶	0.2
NB-Ebc1	Neuroblastoma	51.1	0.30	>1 x 10 ⁻⁵	0.1
CHLA-90	Neuroblastoma	66.2	0.50	>1 x 10 ⁻⁵	0.1
CHLA-136	Neuroblastoma	95.8	0.93	>1 x 10 ⁻⁵	0.1
NALM-6	ALL	12.5	0.07	2.65 x 10 ⁻⁷	4.6
COG-LL-317	ALL	0.1	-0.98	6.49 x 10 ⁻⁹	188.1
RS4;11	ALL	0.7	-0.96	1.47 x 10 ⁻⁷	8.3
MOLT-4	ALL	1.4	-0.92	4.00 x 10 ⁻⁸	30.5
CCRF-CEM	ALL	6.7	-0.33	2.68 x 10 ⁻⁷	4.6
Kasumi-1	AML	2.1	-0.95	1.07 x 10 ⁻⁷	11.4
Karpas-299	ALCL	15.5	0.06	2.93 x 10 ⁻⁶	0.4
Ramos-RA1	NHL	35.1	0.33	7.35 x 10 ⁻⁶	0.2

* (T/C)_{Min}% = Lowest T/C value at any tested concentration of GSK690693;

*** Relative In/Out (I/O): For lines with $T/C_{Min} > input$, equation is $(T/C_{Min} - input)/(100 - input)$. For lines with $T/C_{Min} < input$, equation is $(T/C_{Min} - input)/input$. Relative I/O values close to 0 indicate cytostasis, close to 1 indicate no effect, and close to -1 indicate complete cytotoxicity.

Table II

Activity for GSK690693 Against the PPTP *in Vivo* Panel

Xenograft Line	Histology	P- value	EFS T/C	Median Final RTV	Tumor Volume T/C	P- value	T/C Activity	EFS Activity	Response Activity
BT-29	Rhabdoid	0.457	1	>4	1.05	0.684	Low	Low	Low
KT-14	Rhabdoid	0.24	1	>4	0.78	0.315	Low	Low	Low
KT-12	Rhabdoid	0.005	> 3.8	3.4	0.65	0.077	Low	Int	Int
KT-10	Wilms	0.265	1	>4	0.93	0.684	Low	Low	Low
KT-13	Wilms	0.924	1	>4	0.95	0.971	Low	Low	Low
SK-NEP-1	Ewing	0.819	0.9	>4	1.05	0.497	Low	Low	Low
EW5	Ewing	0.192	1.1	>4	0.75	0.218	Low	Low	Low
EW8	Ewing	0.123	1.6	>4	0.76	0.035	Low	Low	Int
TC-71	Ewing	0.183	0.8	>4	1.29	0.156	Low	Low	Low
CHLA258	Ewing	0.481	1	>4	1.1	0.897	Low	Low	Low
Rh10	ALV RMS	0.207	1.3	>4	0.71	0.023	Low	Low	Low
Rh28	ALV RMS	<0.001	1.7	>4	0.46	<0.001	Low	Low	Int
Rh30	ALV RMS	0.207	1.3	>4	0.85	0.460	Low	Low	Low
Rh30R	ALV RMS	0.261	1	>4	0.94	0.739	Low	Low	Low
Rh41	ALV RMS	0.229	2	>4	0.86	0.579	Low	Low	Int
Rh65	ALV RMS	0.506	0.8	>4	0.98	1.000	Low	Low	Low
BT-28	Medulloblastoma	0.387	0.9	>4	1.28	0.481	Low	Low	Low
BT-45	Medulloblastoma	0.787	1.1	>4	0.95	0.780	Low	Low	Low
BT-50	Medulloblastoma	0.341	> 1.0	3.6	1.01	0.631	Low	NE	Int
BT-44	Ependymoma	0.049	1.5	>4	0.71	0.011	Low	Low	Low
GBM2	Glioblastoma	0.016	1.8	>4	0.79	0.165	Low	Low	Int
BT-39	Glioblastoma	0.237	1.1	>4	0.94	0.218	Low	Low	Low
D645	Glioblastoma	<0.001	1.4	>4	0.64	0.003	Low	Low	Low
D456	Glioblastoma	0.077	1.2	>4	0.84	0.089	Low	Low	Low
NB-1771	Neuroblastoma	<0.001	1.2	>4	0.69	0.182	Low	Low	Low
NB-1691	Neuroblastoma	0.672	1	>4	1.18	0.815	Low	Low	Low
NB-EBc1	Neuroblastoma	0.473	0.7	>4	1.82	0.123	Low	Low	Low

Xenograft Line	Histology	P- value	EFS T/C	Median Final RTV	Tumor Volume T/C	P- value	T/C Activity	EFS Activity	Response Activity
CHLA-79	Neuroblastoma	0.474	0.9	>4	0.99	0.905	Low	Low	Low
OS-1	Osteosarcoma	<0.001	> 1.4	2.8	0.65	0.015	Low	NE	Int
OS-2	Osteosarcoma	0.006	1.5	>4	0.6	0.002	Low	Low	Low
OS-17	Osteosarcoma	0.028	1.3	>4	0.71	0.029	Low	Low	Low
OS-9	Osteosarcoma	0.014	1.4	>4	0.68	0.011	Low	Low	Low
OS-33	Osteosarcoma	<0.001	2.7	>4	0.21	<0.001	Int	Int	Int
OS-31	Osteosarcoma	0.002	1.2	>4	0.82	0.002	Low	Low	Low
ALL-2	ALL B-precursor	0.454	1.5	>25	.			Low	Int
ALL-3	ALL B-precursor	0.199	1.4	>25	.			Low	Low
ALL-4	ALL B-precursor	0.739	1.4	>25	.			Low	Low
ALL-7	ALL B-precursor	0.481	2.3	>25	.			Low	Int
ALL-8	ALL T-cell	0.935	1.2	>25	.			Low	Low
ALL-16	ALL T-cell	0.816	0.7	>25	.			Low	Low
ALL-17	ALL B-precursor	0.841	0.8	>25	.			Low	Low
ALL-19	ALL B-precursor	0.513	1.4	>25	.			Low	Low