SUPPLEMENTARY METHODS

Pharmacodynamic assessment methods

Pharmacodynamic and molecular profiling analyses were conducted under non-GLP conditions at Exelixis, Inc.

Plasma biomarker analyses

Fasted plasma insulin, glucose, insulin growth factor-binding protein 2 (IGFBP-2) and regulated on activation normal T cell expressed and secreted (RANTES) were evaluated in plasma. Blood samples were collected from patients receiving 30 to 900mg of SAR245408 (three patients per cohort except six patients for 600mg) on Days 1, 8 and 21 of the 21/7 regimen and fasting overnight prior to dosing and through the two-hour timepoint before breaking fast. Blood was collected in 8ml BD Vacutainer CPT™ tubes with sodium citrate (Becton-Dickinson #367835), and plasma was obtained after centrifugation at room temperature (20–30 minutes, 1500–1800 RPM), snap-frozen in liquid nitrogen or on dry ice, and stored at −70°C or below. Plasma samples from selected patients were analyzed using commercial ELISA assays: Mercodia Insulin assay (#10-1113-01), BioVision Glucose assay (#K606-100), R&D Systems VEGF-A assay #DVE00), RayBiotech Inc. IGFBP-2 assay (Cat# ELH-IGFBP2-001), and R&D Systems RANTES assay (Cat# DRNOOB).

Pharmacodynamic analysis of peripheral blood mononuclear cell (PMBCs) lysate

PBMCs were collected on site using 8ml BD Vacutainer CPT™ tubes with sodium citrate (Becton-Dickinson #367835), rinsed in room-temperature phosphate-buffered saline, and lysed in freshly prepared RIPA buffer cocktail before being stored at -70°C or below. Upon
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shipment to the sponsor, lysates were thawed on ice, clarified (by centrifugation at 10K RPM, 4°C for 10 min) and 25μg protein per sample resolved using electrophoresis on Bio-Rad Cetrieno gels, transferred to nitrocellulose membranes (BioRad #162_0234) and analyzed by western blotting. Blots were blocked at RT in 5% non-fat dry milk, 1x TBS, 0.1% Tween-20 for 1h, except for pPRAS40 in 3% dry milk/PBS with 0.05% Tween-20. Blots were then incubated with the following primary antibodies overnight at 4°C in 5% BSA, 1x TBS, 0.1% Tween 20: pAKT5473 (CST #9271 at 1:500), pPRAS40T246 (Upstate #07-888 at 1:1000) both in 3% milk/PBS/0.05%Tween-20; pGSK3βS9 (CST #9336 at 1:500), p4EBP1T37/T46 (CST #9459 at 1:500), Ezrin (CST #3145 at 1:2000), and GAPDH (CST #2118 at 1:10000). After washes, secondary antibody (Anti-Rabbit IgG HRP Linked, CST #7074 at 1:10000 for GAPDH and at 1:5000 for Ezrin and the various phospho-proteins) was added for 1 hour at RT, before washing and signals were revealed on film, and phospho-protein readouts analyzed with ImageQuant software as follows: phospho-protein signal was normalized to the same-lane loading control (GAPDH or Ezrin), then modulation assessed relative to Day 1 pre-dose. Gel-specific controls were A549 NSCLC cell line lysates and healthy donor PBMC lysates. For several patients analyzed most recently, GAPDH was detected (Advanced ImmunoChemical #2-RGM2 1:10,000; Secondary Anti-Mouse IRDye 800CW Antibody: LI-COR, #926-32210, 1:10,000) using the Odyssey system.

Pharmacodynamic analysis of hair samples

Hairs were collected either from the scalp (head hair) or eyebrows using blunt-nosed forceps with analysis done with all hair samples collected from the same site (head or eyebrows) for each patient. Samples containing a hair sheath were trimmed to approximately 1–2cm in length containing the sheath and bulb region, placed in 1.5ml
cryotubes with screw top closure and immersed completely in zinc fixative for 48h (BD Pharmingen, #550523) followed by transfer into 70% ethanol, before storing at -70°C or below. For embedding, frozen samples were thawed on ice, and 2–4 hairs per patient embedded together in Histogel support matrix and processed into a paraffin block. Serial 5μm thick sections were prepared and placed onto positively charged Shandon Colormark Plus microscopy slides (#B9992010CS). Since phosphoprotein expression varies across the length of hair sheaths, every 3rd, 4th, or 5th section were placed on microscopy slide and stained for protein detection, allowing sequential analysis for 3, 4, or 5 readouts across the total length of a hair sheath. Phospho protein were detected using primary antibodies pAKT^T308 (CST #4056, dilution 1:200), pAKT^S473 (CST #3787, dilution 1:25), p4EBP1^T70 (CST #9455, dilution 1:50), pPRAS40^T246 (Biosource #44-1100G, dilution 1:200), and pS6^S240/S244 (CST #2215, dilution 1:100). Sections were then incubated with Alexa-594 goat anti-rabbit secondary antibody (Molecular probes, #A11037, dilution 1:200). Stained sections were mounted in Fluorescent Mounting Medium (DAKO #S3023) containing DAPI (Invitrogen, #D3571) as a nuclear counterstain.

Following staining, all sections were assessed for presence of tissue and staining quality, and up to 40 fields spaced across the entire length of the hair sheath were captured at 400x magnification using an AxioImager A1 microscope equipped with 40x/0.95 Corr Plan-Apochromat objective, high resolution AxioCam MRm camera, and Axiovision software (Zeiss, Thornwood, NY). Digital images of the cross-sectional hair sheaths were quantified using a MetaMorph based strategy (Molecular Devices) using automated macros to enable quantification of fluorescently labeled cellular components after grey scale conversion of blue, green, and red color channels to achieve a linear 1–255 intensity scale. A consistent
threshold grayscale value for each color channel above which specific pixel recognition was achieved was applied to each image. The following three parameters were calculated:

Protein Expression = Target Protein Pixel Area / DAPI Pixel Area (%), Protein Abundance = \( \Sigma \) Intensity of Target Pixels / Target Pixel Area (Intensity/Pxl), and a Normalized Intensity Score = \( \Sigma \) Intensity of Target Pixels Pixels x (Target Pixel Area / DAPI Pixel Area). Mean and SD values for hair samples collected between C1D1 pre-dose to D85 pre-dose were calculated, followed by determination of percent decrease in biomarker expression in hair samples collected post-dose compared with baseline. Statistical Analysis employed two-tailed student t-test analysis with \( p<0.05 \) to be considered statistically significant. Bonferroni adjustment was applied if multiple comparisons against a single baseline sample were performed.

**Pharmacodynamic analysis of skin biopsies**

Cryopreserved skin biopsy samples were received frozen in O.C.T™ (#4583, Sakura) on dry ice and stored at -70°C or below prior to sectioning. For immunofluorescence analysis, samples were serially sectioned at 10 micron thickness. Twenty serial sections were obtained from each sample, with slide 1 stained with H&E to assess tissue quality and subsequent slides stained to detect expression of phosphorylated AKT\(^{T308}\) (CST #4056, 1:200), AKT\(^{S473}\) (CST #3787, 1:25), 4EBP1\(^{T70}\) (CST #9455, 1:50), PRAS40\(^{T246}\) (Biosource #44-1100G, dilution 1:200), S6\(^{S240/244}\) (CST #2215, dilution 1:100), MEK\(^{S217/S221}\) (CST #9121, dilution 1:200), and ERK\(^{T202/Y204}\) (CST #4376, dilution 1:50) overnight at 4°C. Skin sections were then incubated in goat anti-rabbit Alexa 594-conjugated secondary antibody (Molecular Probes #A11037, dilution 1:200), with DAPI (Invitrogen #D3571) as nuclear counterstain. TUNEL was carried out using an in situ cell death detection kit according to the
manufacturer’s instruction (Roche #11 684 817 910). Following staining, all sections were assessed for tissue and staining quality and 12 non-overlapping representative fields were captured at 400x magnification using an AxioImager A1 microscope equipped with 40x/0.95 Corr Plan-Apochromat objective and high resolution AxioCam MRm camera using Axiovision software (Zeiss, Thornwood, NY). Digital images of the epidermal cell layer (ROI) were quantified using a MetaMorph based strategy (Molecular Devices). Briefly, individual images of the epidermis were analyzed using automated macros (MetaMorph journals) to enable quantification of fluorescently labeled cellular components as detailed above for hair. Mean and SD values for baseline and post-dose samples were calculated, followed by the percent decrease in biomarker expression from skin collected post-dose compared with baseline.

Statistical Analysis employed two-tailed student t test analysis with p<0.05 to be considered statistically significant. Bonferroni adjustment was applied if multiple comparisons against a single pre-dose control sample were performed.

**Pharmacodynamic analysis of tumor tissue biopsies**

Cryo-preserved tumor biopsy samples were received frozen in O.C.T™ (#4583, Sakura) on dry ice and stored at -70°C or below prior to sectioning. For immunofluorescence analysis, samples were serially sectioned at 10 microns and 20 serial sections were processed and stained with antibodies as described for skin biopsies. All protocols were executed in dual-probe fashion to detect phosphorylated marker and Ki67 nuclear antigen (Vector, #VP-K452, clone MM1, dilution 1:100) to delineate tumor cell ROI vs normal tissue compartments. For detection of pERK and total ERK, 20 serial sections were obtained from each sample, with slide 1 stained with H&E to assess tissue quality and subsequent slides dual-probe stained to detect expression of phosphorylated ERKT202/Y204 (CST #4376, dilution 1:50) and total ERK.
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(CST, #9107, dilution 1:100) in the same section. After overnight incubation with primary antibody at 4°C, sections were incubated in goat anti-rabbit or goat anti-mouse Alexa 488-conjugated secondary antibody (1:200 dilution; Molecular Probes #A11037 and #A11032) with DAPI (Invitrogen #D3571) as nuclear counterstain. TUNEL was carried out using an in situ cell death detection kit according to the manufacturer’s instruction (Roche #11 684 817 910).

Following staining, all sections were assessed for tissue and staining quality and up to 28 non-overlapping representative fields were captured at 400x magnification using an AxioImager A1 microscope equipped with 40x/0.95 Corr Plan-Apochromat objective and high resolution AxioCam MRm camera using Axiovision software (Zeiss, Thornwood, NY). Digital images of the epidermal cell layer (ROI) were quantified using a MetaMorph based strategy (Molecular Devices). Digital images of the cell layer (ROI) were quantified using a MetaMorph based strategy (Molecular Devices) or an automated approach using Definiens XD Ecognition. Briefly, a delineation of tumor cell vs normal tissue compartments was either performed using the higher proliferative index of tumor cells (Ki67) as a guide (Metamorph), or in the case of the Definiens-based analysis, proliferative rate (Ki67), cell and nuclear shape were used to identify tumor cells. Images were then analyzed using automated macros (MetaMorph journals) or rulesets (Definiens). In both cases, macros and rulesets were designed to enable quantification of fluorescently labeled cellular components after grayscale conversion of blue, green, and red color channels to achieve a linear 1–255 intensity scale. A consistent threshold grayscale value for each color channel above which specific pixel recognition was achieved was applied to each image. The following three parameters were calculated: Protein Expression = Target Protein Pixel Area / DAPI Pixel Area.
(\%), Protein Abundance = \Sigma \text{Intensity of Target Pixels} / \text{Target Pixel Area} (\text{Intensity}/\text{Pxl}), and a Normalized Intensity Score = \Sigma \text{Intensity of Target Pixels}. Statistical Analysis employed two-tailed student t test analysis with p< 0.05 to be considered statistically significant. Bonferroni adjustment was applied if multiple comparisons against a single baseline sample were performed.

**Molecular profiling of archival or fresh tumor tissue**

PTEN expression was evaluated using immunohistochemistry in formalin fixed, paraffin-embedded tissue sections (5 micron) with anti-PTEN antibody (NCL-PTEN, clone 28H6, diluted 1:300, Novocastra Laboratories Ltd) utilizing standardized procedures. Briefly, prior to immunostaining, microwave-based antigen retrieval step in deparaffinized tissue sections was performed in Citrate Buffer (Lab Vision #AP-9003) for a total of 5 min. After a 20-minute cooling period, endogenous peroxidase activity was blocked for 5 min in KPL buffer (KPL #71-00-10). After 30-minute incubation with CAS block (Zymed #00-8120) specimens were incubated with primary antibody overnight at 4°C and detection of primary antibody performed using the appropriate Dako EnVision System (Dako #K4001) and 3,3V-diaminobenzidine as a substrate. Slides were counterstained with hematoxylin (Richard-Allan Scientific, #7221). Each staining procedure contained a positive (IBS 03-08011 carotid metastasis) and negative IgG control. Predominantly nuclear over cytoplasmic PTEN staining in tumor and non-neoplastic tissues was observed and quantified. PTEN staining in the non-neoplastic normal vascular endothelium and extratumoral stromal cells served as the internal positive control for each patient. PTEN stained tumor samples were scanned onto Aperio XT digital slide scanner and analyzed using Aperio algorithm to quantify number of positive tumor nuclei (%) and intensity score of positive tumor nuclei (using a standardized
scoring scale of 0= negative, 1= weak, 2= moderate, 3= strong based on pixel intensities) followed by calculating an H-Score for each sample applying the following formula: $H$-Score = $1 \times (%$ weak nuclear staining) + $2 \times (%$ moderate nuclear staining) + $3 \times (%$ strong nuclear staining).

Sequencing for alterations in genes relevant to the target profile of SAR245408 analysis was performed as follows. Genomic DNA was isolated from formalin-fixed paraffin-embedded biopsies (Sjøholm, et al. Cancer Epidemiol Biomarkers Prev 2005;14:251–255) using the modified QIAquick DNA extraction protocol or the PicoPure DNA extraction kit (Molecular Devices, Sunnyvale, CA). For fresh frozen tumor tissue, similar methods were used to isolate DNA from OCT sections. Genomic DNA quality was analyzed by gel check. DNA was amplified by PCR using primers flanking known mutational hotspots. The resulting PCR products were cleaned up with ExoSAP-IT (USB, Cleveland, OH) according to manufacturer instructions and sequenced bidirectionally using the same PCR primers as the sequencing primers. Sequencing reaction products were analyzed on automated ABI 3730XL sequencers (Applied Biosystems, Foster City, CA), and traces were analyzed using the Mutation Surveyor software package (SoftGenetics, State College, PA) and manual inspection of the traces. In general, direct Sanger sequencing is capable of detecting homozygous and heterozygous somatic mutations in samples with >20% and >40% tumor cells, respectively. When direct Sanger sequencing was not feasible, 454 sequencing was utilized. Exons and adjacent splice sites of the requested genes were amplified by regular PCR. The amplicons were sequenced using the Roche 454 FLX genome sequencer and analyzed using AVA software. The range of quality reads was recorded for each amplicon. In general, the noise level was about 1.0% of total reads for each amplicon, so mutations could be detected if they exceeded the 3.0%
threshold. Identified mutations were confirmed by independent PCR amplification and Sanger sequencing. Quantitative polymerase chain reaction (PCR) was used to evaluate PIK3CA, EGFR, MET and KIT gene amplification in some samples. RNase P forward and reverse primers and probes were purchased as a part of the TaqMan RNase P Control Reagents Kit (Applied Biosystems). PCR reactions were carried out on the ABI PRISM 7900 HT system using TaqMan Universal PCR Master Mix (Applied Biosystems). All samples were analyzed in triplicate, and the relative target copy number was derived by standardizing the input DNA to two reference genes (ALB, chromosome 4, and RNase P, chromosome 14). Dilution of cancer cells with non-cancer cells could have led to an underestimate of the degree of amplification in some samples.

For PTEN and MGMT promoter methylation analysis, genomic DNA was converted using the QIAGEN EpiTect Bisulfite kit and analyzed by methylation-specific PCR, using primers specifically designed to amplify either methylated or unmethylated sequence.

**Pharmacokinetic assessment methods**

Concentrations of SAR245408 in both plasma and urine below the lower limit of quantitation at pre-dose timepoints were zero’d for pharmacokinetic (PK) analyses. Descriptive statistics of plasma concentrations based on the nominal collection times and PK parameters were summarized by cohort using WinNonlin Professional 5.2 (Pharsight Corp., Mountain View, CA) and Microsoft Excel 2003 (Microsoft Corporation, Redmond, WA). Individual and mean concentration–time profiles and exposure plots were generated using Sigma Plot v11 (Systat Software Inc., Chicago, IL). Individual concentration–time data for patients without dose modifications (including dose reduction, dose holding or dose
escalating to higher doses) were subjected to non-compartmental analysis using WinNonlin Professional 5.2. Actual timepoints and doses were used for the non-compartmental analyses. For timepoints without actual dosing time or PK sampling time, nominal times were used. All calculations were performed prior to rounding the values.

The following PK parameters were estimated and reported for SAR245408.

Terminal half-life ($t_{\frac{1}{2},z}$) was calculated as $t_{\frac{1}{2},z} = \ln(2)/k_{el}$, where $k_{el}$ was the first-order terminal rate constant estimated via linear regression of log-linear decay phase, expressed in hours. Half-life values were excluded from summary statistics if the %AUC$_{0\text{-}\infty}$ extrapolated was >30%.

The observed time ($t_{\text{max}}$) for SAR245408 at which the observed maximum concentration ($C_{\text{max}}$) occurred was recorded and expressed in hours.

$C_{\text{max}}$ for SAR245408 was recorded and expressed as ng/ml.

Dose normalized for $C_{\text{max}}$ and AUC$_{\text{last}}$ for SAR245408 ($C_{\text{max}}/D$ and AUC$_{\text{last}}/D$) were expressed as ng/ml/mg/kg for $C_{\text{max}}/D$, and ng*hr/ml/mg/kg for AUC$_{\text{last}}/D$.

The area under the plasma concentration–time curve parameters AUC$_{0\text{-}24}$ and AUC$_{\text{last}}$ for SAR245408 were estimated using the linear/log trapezoidal method from time 0 to 24 hours (or dosing interval) or time of the last observable value (or dosing interval), respectively, and were expressed as ng*hr/ml.

The area under the plasma concentration–time curve from time 0 to infinity (AUC$_{\infty}$) for SAR245408 was estimated as the sum of the corresponding AUC$_{\text{last}}$ and the predicted $C_{\text{last}}/k_{el}$ values: AUC$_{\infty} = AUC_{\text{last}} + \text{predicted } C_{\text{last}}/k_{el}$, unit as ng*hr/ml.

Total apparent plasma clearance (Cl/F) for SAR245408 was calculated as Dose/AUC$_{0\text{-}24}$ on Day 21 or 28.
SAR245408 accumulation ratio (AR) on Cycle 1 Day 21 or Cycle 1 Day 28 based on C_{max} (ARC_{max}) was calculated as \( ARC_{max} = \frac{C_{max} \text{ Day 21 or } C_{max} \text{ Day 28}}{C_{max} \text{ Day 1}} \).

SAR245408 AR on Cycle 1 Day 21 or on Cycle 1 Day 28 based on AUC_{0-24} (AR AUC_{0-24}) was calculated as \( AR \text{ AUC}_{0-24} = \frac{AUC_{0-24} \text{ Day 21 or } 28}{AUC_{0-24} \text{ Day 1}} \).

Amount of SAR245408 excreted unchanged in urine was calculated as concentration in urine * total urine volume collected up to 24 hours post-dose in Cycle 1 Day 20 or Cycle 1 Day 28.

The percentage of SAR245408 excreted unchanged in urine was calculated as (amount excreted unchanged in urine/total dose given) * 100.