Title

First in Human, Phase 1, Dose Escalation Pharmacokinetic and Pharmacodynamic Study of the Oral Dual PI3K and mTORC1/2 Inhibitor PQR309 in Patients with Advanced Solid Tumors (SAKK 67/13)

Authors & Affiliation

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Abstract

Background: PQR309 is an orally bioavailable, balanced pan-PI3K, mTORC1 and mTORC2 inhibitor.

Patients and Methods: This is an accelerated titration, 3+3 dose-escalation, open label phase I trial of continuous once daily (OD) PQR309 administration to evaluate the safety, pharmacokinetics (PK) and pharmacodynamics (PD) in patients with advanced solid tumours. Primary objectives were to determine the maximum tolerated dose (MTD) and recommended phase 2 dose (RP2D).

Results: 28 patients were included in 6 dosing cohorts and treated at a daily PQR309 dose ranging from 10 to 150mg. Common adverse events (AE) (≥30% patients) included fatigue, hyperglycaemia, nausea, diarrhea, constipation, rash, anorexia and vomiting. Grade (G) 3 or 4 drug-related AE were seen in 13 (46%) and 3 (11%) patients, respectively.

DLT was observed in two patients at 100mg OD (>14 day interruption in PQR309 due to G3 rash, G2 hyperbilirubinaemia, G4 suicide attempt; dose reduction due to G3 fatigue, G2 diarrhea, G4 transaminitis) and one patient at 80mg (G3 hyperglycemia >7 days). PK shows fast absorption (T_{max} 1-2h) and dose proportionality for C_{max} and AUC. A partial response in a patient with metastatic thymus cancer, 24% disease volume reduction in a patient with sinunasal cancer, and stable disease for over 16 weeks in a patient with clear cell Bartholin's gland cancer were observed.

Conclusion: The MTD and R2PD of PQR309 is 80mg PO OD. PK is dose-proportional. PD shows PI3K pathway phosphoprotein downregulation in paired tumor biopsies. Clinical activity was observed in patients with and without PI3K pathway dysregulation.

Clinical trial registration: ClinicalTrials.gov # NCT01940133

Introduction

The phosphatidylinositol-3-kinase (PI3K) and mammalian target of rapamycin (mTOR) signaling cascade serves physiological and pathophysiological cell functions and is of major importance in cancer and inflammatory disease. As a key downstream effector of receptor tyrosine kinases (RTKs) and G protein coupled receptors (GPCRs), PI3K activation initiates a signal transduction pathway that stimulates glucose metabolism, cell proliferation, and survival [1–9]. One of the principal downstream effectors of PI3K is mTOR. mTOR also integrates growth signals that are independent of PI3K activation [6]. Dysregulation of the PI3K/mTOR pathway is associated with many cancers and may occur through several mechanisms including (i) activation of the p110α subunit (PI3KCA); (ii) activation of upstream receptor tyrosine kinases; (iii) constitutive recruitment and activation by Ras oncogene mutants; (iv) loss or inactivating mutations of Phosphatase and tensin homolog (PTEN); or (v) overexpression and activating mutations of downstream kinases (e.g., Akt) [2,6]. In addition, dysregulation of the PI3K/mTOR pathway has been implicated in chemotherapy resistance [2,6,9–12].

To date, PI3K and PI3K/mTOR inhibitors have demonstrated clinical efficacy in cancer patients with or without PIK3CA [13] or PTEN aberrations. Idelalisib, a selective inhibitor of PI3Kδ is licensed for use in chronic lymphocytic leukaemia and follicular lymphoma [14–16]. Everolimus, a selective inhibitor of mTORC1, is licensed for use in advanced breast cancer, neuroendocrine tumors, and renal cell carcinoma. Although these clinical data are encouraging, clinical resistance to kinase inhibitors occurs either due to novel mutations within the targeted kinase, or to other compensatory mechanisms [17–19]. In particular, it has been shown that idelalisib resistance is due to increased expression of PI3Kα [20]. Alternatively, inhibiting all PI3K isoforms results in upregulation of the mTOR pathway or inactivation of PTEN, accompanied by resistance to these agents [21]. Persistent mTOR activation has been detected in patients with PIK3CA-inhibitor resistant tumors. Thus, targeting two nodal points within a pathway may reduce the probability of resistance [21]. Dual inhibition of PI3K and mTOR is therefore a promising strategy for anticancer therapy.

PQR309 (Piqur Therapeutics AG, Basel, Switzerland) is an oral pan-class I PI3K inhibitor that selectively targets all four isoforms of class I PI3K (α, β, γ, δ), with a balanced activity against mTOR. It is equipotent against $p110\alpha^{H1047R/E542K/E545K}$ somatic mutations often observed in human cancers[22]. PQR309 demonstrates anti-proliferative activity in a variety of cell lines with and without inappropriate PI3K pathway activation [23–27].

The primary objectives of this first-in-human, phase 1, dose-escalation study were to assess the safety and tolerability and determine the MTD and RP2D of oral PQR309 with once-daily continuous dosing in patients with advanced solid tumors. Secondary objectives included characterization of the pharmacokinetics (PK) and pharmacodynamics (PD), and preliminary assessment of anti-tumor activity of PQR309.

Patients and Methods

Study design

This was a multicenter, open-label first-in-human trial. Based on the No-Observed-Adverse-Effect-Level (NOAEL) in dogs of 4mg/kg, the starting dose in humans was 10mg. An accelerated modified "3+3" dose-escalation design was used. Dose level 1 and 2 enrolled a single patient. If a drug-related toxicity ≥ grade 2 occurred, two additional patients were to be enrolled at the same dose level and then the trial would continue as a classical 3+3. From dose level 3 and thereafter, the classical 3+3 design was used. Doses were increased by 100% between dose-levels until dose level 4. After dose level 4 or the first toxicity ≥ grade 2, subsequent dose levels could increase between 30-100%, according to the type and grade of toxicity after discussion with the independent data safety monitoring board (IDSMB). In dose levels 2 to 4, the administered dose was adjusted according to weight (75% dose if < 60kg, 125% dose if > 80kg). Eligible patients received once daily oral PQR309 capsules continuously on a 21 day cycle until progression, unacceptable toxicity, investigator judgement, or withdrawal of consent. The study protocol was approved by ethics committees and regulatory authorities of all institutions and all participants provided written informed consent.

Patients elligibility

The study enrolled adult patients (age ≥ 18 years) with a histological or cytologically confirmed diagnosis of advanced solid tumor and evidence of tumor progression with measurable or evaluable disease. The inclusion criteria were updated after recruitment of the four initial cohorts to require tumors accessible to biopsy. Detailed in- and exclusion criteria are shown in supplementary table 1.

Dose limiting toxicity, maximum tolerated dose, and management of toxicity

DLTs were defined as any of the following: grade 4 neutropenia for > 7 days, febrile neutropenia, grade 4 thrombocytopenia, grade 4 non-hematological toxicity (e.g. hyperglycemia > 27.8 mmol/L) or grade 3 lasting > 7 days (unless controlled with supportive care), treatment delay > 14 days due to unresolved toxicity, or non-hematological toxicity ≥ grade 2 deemed dose limiting by the IDSMB. DLTs were based on adverse events observed during the first cycle (21 days). The MTD was defined as the highest dose level at which ≤ 1 out of 6 patients experience a DLT.

Safety & efficacy assessments

Adverse events were graded using the National Cancer Institute (NCI) Common Toxicity Criteria for Adverse Events (CTCAE) version 4.03. Efficacy parameters were defined using the Response Evaluation Criteria in Solid Tumors (RECIST version 1.1). Full disclosure of assessments is available in supplementary material & methods.

Results

Patient characteristics

Twenty-eight patients (20 female, 8 male) were treated between January 2014 and February 2015 at six centers (Switzerland, the United Kingdom and Spain) (Table 1). Patients were enrolled into six dosing cohorts (10mg n=1; 20mg n=1; 40mg n=4; 80mg n=9; 100mg n=7; 120mg n=6), with dose

adjusted for body weight in the first four cohorts (Table 2). The median age of patients was 58 years (range 21-75). The most frequent primary tumor types were colorectal cancer (n=7) and ovarian cancer (n=6). The median number of lines of prior treatment was 4 (range 0-9; Table 1). Fourteen patients (50%) discontinued trial treatment due to progressive disease. Five patients discontinued due to adverse events (18%), six due to withdrawal of consent (21%; toxicity and intolerance of supportive therapies were given as reason), one due to death secondary to cancer, and one was withdrawn by the investigator. One patient left the trial after 21 cycles and continued PQR309 treatment within the framework of a compassionate use program.

Adverse events

Fatigue was the most common adverse event in this trial (Table 3). 26 of 28 patients (93%) experienced at least one episode of fatigue. Of those, 3 (11%) had fatigue grade 3-4.

Hyperglycemia was observed in 25 (89%) patients, 7 (25%) had G3-4 hyperglycemia. Glucose levels, PQR309 dosage and anti-hyperglycemic therapy are presented graphically (Suppl. Figure 2). Hyperglycemia onset was 7-14 days after commencing PQR309. The median time to normalization of blood glucose levels after stopping PQR309 was 7 days. Hyperglycaemia was managed with metformin, sulfonylureas, SGLT-2 inhibitors, and insulin. Insulin was required in 5 of the 7 patients with G3-4 hyperglycemia.

Anorexia occurred in 15 patients (54%) and weight loss >5% in 13 (46%) participants. Weight loss was reversible after stopping PQR309.

8 (29%) patients developed a maculopapular rash which was G3-4 severity in 5 patients. Corticosteroids were effective and induced rapid remission of the rash.

Depression was observed in 6 (21%) patients. In 1 patient (4%) a psychotic episode with suicide attempt was witnessed a few days after stopping the trial drug. No Grade 5 toxicities were observed.

Dose-limiting toxicities

Dose-escalation continued to 120mg once daily. No DLT was observed in the initial four dose-escalation cohorts up to 80mg daily (Table 2). Although no formal DLT was declared in the 120mg cohort, the investigators judged this dose to be above the maximum tolerated dose due to the frequency of Grade 3 AEs including hyperglycemia in 4 out of 6 patients; rash in 2 patients; and fatigue, anemia, nausea, vomiting, diarrhea, broncho-pulmonary infection, hypoxia, ALT increase, AST increase, ALP increase, headache, and hypertension in 1 patient. Based on this, an additional cohort was accrued at the 80mg flat dose level. No DLT occurred. As specified by the protocol an intermittent dose level of 100mg daily was opened. In this cohort, a G3 rash and G4 suicide attempt in a patient with colorectal cancer was considered a DLT. A second DLT in the 100mg cohort occurred in a patient with ovarian cancer at the same dose level who experienced G3 fatigue, G3 diarrhea and G3 elevation of liver enzymes. Given 2 DLTs in the 100mg cohort, three additional patients were enrolled in the 80mg cohort, of whom one patient experienced a DLT (G3 hyperglycemia for more than 7 days). 80mg was declared the maximum tolerated dose and the recommended phase 2 dose (RP2D) for continuous daily dosing of PQR309.

Response

Twenty-four patients were evaluable for response by radiological assessment of target lesions using RECIST v1.1 (Table 2). A partial response was observed in one patient. Best response of stable disease or progressive disease was observed in nine and fourteen patients, respectively. The median duration of treatment was 41.5 days (range 12-446). Two patients were on treatment for over 100 days at 100mg daily; a patient with thymic carcinoma with a known *RICTOR1* amplification with a partial response on imaging (-42%, ongoing on day 705); and a patient with Bartholin's gland carcinoma with a SMARCB1 mutation and stable disease (152 days).

Pharmacokinetics

Absorption was moderately fast to fast. The peak plasma concentration, $C_{max,}$ was generally reached between 1 to 2 hours after oral administration of PQR309, except for some patients at 50, 80 and 120 mg PQR309 where C_{max} was reached at 6 to 24 hours after oral administration of PQR309. The average peak concentration (C_{max}) and exposure (AUC_{last}) increased with increasing dose levels of PQR309 in a roughly dose proportional manner; with a minimum of 49.9 ng/mL and 273 h*ng/mL (15 mg) and a maximum of 998 ng/mL (150 mg) and 12600 h*ng/mL (120 mg), for C_{max} and AUC respectively. The variability per group in the PK parameters C_{max} and AUC, evaluated by %CV, varied from 13 to 80%. After repeated administration of PQR309 most patients showed higher plasma values after 21 days of treatment of PQR309 as compared to the pre-dose levels on Day 2 (=24-hour post first dose on Day 1), independent of dose level. Average accumulation ratios when comparing pre-dose Day 22 (Cycle 2 Day 1) to pre-dose Day 2 (=24 hours post first dose on Day 1, Cycle 1) varied from 1.0 to 9.7, and were not dose-related. $T_{1/2}$ of PQR309 is estimated to be around 40 hours from the 0-24 hour profile carried out in this study.

Pharmacodynamics

17 patients had at least one tumor biopsy taken for pharmacodynamic analysis, although not all samples were sufficient for all planned translational assays.

The analysis of 88 PI3K-related mRNAs (Suppl. Figure 1) in PTB of 6 patients showed a non-significant threefold upregulation of PDGFRA (Suppl. Figure 3). No consistent up- or downregulation of the remaining 87 mRNAs was detected. Thus, there is no transcriptional feedback regulation after 21 days of therapy with PQR309.

Samples from 13 patients were eligible for the analysis of phospho-proteins. Table 4 summarizes key characteristics of the 13 eligible paired biopsies. Changes in the level of phosphorylation are demonstrated in Figure 1 and supplementary Figure 4. Akt phosphorylation sites (Thr308 and

Ser473), p-mTOR, p-S6-RiboProtein, p-AMPKa, p-PRAS40, p-GSK3b, p-Bad, p-RSK1, p-PTEN and p-Erk1/2 were significantly downregulated in comparison to baseline ($p<0.05$, Wilcoxon signed-rank test). p-4E-BP1, p-GSK3a and p-PDK1 showed a non-significant trend to reduced phosphorylation. Patients with tumor reduction by radiological assessment had stronger p-Akt Thr308, p-mTOR Ser2481 and phospho-S6-RiboProtein Ser235/236 (figure 1, suppl. figure 4) suppression than those with tumour increase ($p<0.05$, Mann-Whitney test). Importantly, MAPK activity (Erk1/2 phosphorylation) was the same in patients with tumor shrinkage as compared to those with tumor growth.

The results of the analysis of immune infiltrates in tumor biopsies are shown in Figure 2. No significant change was observed in any of the T cell subpopulations assessed.

Discussion

This first-in-human trial investigated the tolerability and RP2D of PQR309, an oral dual inhibitor of pan-PI3K and mTORC1/2. The main adverse events were fatigue, hyperglycemia, loss of appetite and rash. Six patients (21%) had depression, and amnesia was recorded in one patient (4%). The profile of AEs was broadly similar to that of other pan-PI3K inhibitors such as Buparlisib (NVP-BKM120) or dual inhibitors such as NVP-BEZ235 [28]. The MTD and RP2D of PQR309 was defined as 80mg continuous once daily in advanced solid cancers. Clinical activity including a partial response was observed in patients with and without known PI3K pathway dysregulation. The pharmacokinetic profile suggests dose proportionality and a half-life of 40 hours. Based on the observed toxicity profile and the PK data, alternating dose schedules of PQR309 or 2 days on / 5 days off regimens should be evaluated.

NGS of tumor tissue identified a range of mutations that reflect the known heterogeneity of advanced cancers. Although it is rational to hypothesize that tumours harbouring activating PI3K or mTOR mutations respond better to PI3K-mTOR inhibitors than tumours without, only the BELLE-2 trial has been able to assign a predictive value to such mutations [13]. In the present trial, one patient with

RICTOR amplification [29] and one with an activating *PI3K* mutation (*PIK3CA* p.Glu545Lys) derived benefit from the trial medication. The overall predictive value of PI3K pathway mutations remains unclear. No significant up- or downregulation of PI3K related mRNAs was observed although there was a trend for upregulation of PDGFRA after 21 days of therapy with PQR309.

Exposure to PQR309 significantly downregulated the signalling activity of several PI3K-mTOR associated phospho-proteins, indicating that PQR309 effectively inhibits the intended targets in patients. Consistent with previous preclinical data, PQR309 can also inhibit Erk1/2 signalling in patients. The sample size (1-6 patients per dose level) was too small to show dose-dependent downregulation of PI3K/Akt/mTOR signaling. However, a more pronounced downregulation of p-Akt Thr308, p-mTOR Ser2481 and p-S6-RiboProtein Ser235/236 was observed in those patients whose tumour size diminished while on therapy. Whether this correlates with a higher baseline activation of PI3K/mTOR or is due to a stronger inhibitory effect of the drug in responding patients cannot be determined from the experimental data.

PI3K signalling is involved in the activation of T-cells. There was no evidence that therapy with a dual PI3K-mTOR inhibitor induced immunosuppression in this trial. The analysis of immune infiltrates showed no downregulation of CD8-positive cytotoxic T cells or upregulation of regulatory T cells.

This trial supports further clinical investigation of PQR309 which continues in phase I and II trials including solid tumors with activating *PI3K* mutations (alternative dose scheduling and more intensive PK testing (NCT02850744), lymphoma (NCT02249429), glioblastoma multiforme (NCT02850744), and CNS lymphoma (NCT02669511). The cytostatic nature of PI3K/mTOR inhibitors supports combination therapy approaches and a Phase I/II clinical trial of PQR309 in combination with eribulin is underway in patients with metastatic HER2 negative and triple negative breast cancer (NCT02723877).

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Source of Founding

This clinical trial was fully funded by Piqur Therapeutics.

Role of the Founding Source

PIQUR Therapeutics provided financial support for the study and participated in the design, study conduct and data analysis. PIQUR Therapeutics was involved in review and approval of the manuscript.

Conflicts of interest

NC, VC, MS, SD and RH: Employees of Piqur.

References

[1] Yuan TL, Cantley LC. PI3K pathway alterations in cancer: variations on a theme. Oncogene 2008;27:5497–510. doi:10.1038/onc.2008.245.

[2] Courtney KD, Corcoran RB, Engelman JA. The PI3K pathway as drug target in human cancer. J Clin Oncol 2010;28:1075–83. doi:10.1200/JCO.2009.25.3641.

[3] Banham-Hall E, Clatworthy MR, Okkenhaug K. The Therapeutic Potential for PI3K Inhibitors in Autoimmune Rheumatic Diseases. Open Rheumatol J 2012;6:245–58. doi:10.2174/1874312901206010245.

[4] So L, Fruman DA. PI3K signalling in B- and T-lymphocytes: new developments and therapeutic advances. Biochem J 2012;442:465–81. doi:10.1042/BJ20112092.

[5] So L, Yea SS, Oak JS, Lu M, Manmadhan A, Ke QH, et al. Selective inhibition of phosphoinositide 3-kinase p110 ?? preserves lymphocyte function. J Biol Chem 2013;288:5718–31. doi:10.1074/jbc.M112.379446.

[6] Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. Nat Rev Cancer 2009;9:550–62. doi:10.1038/nrc2664.

[7] Brana I, Siu LL. Clinical development of phosphatidylinositol 3-kinase inhibitors for cancer treatment. BMC Med 2012;10:161. doi:10.1186/1741-7015-10-161.

[8] Rodon J, Dienstmann R, Serra V, Tabernero J. Development of PI3K inhibitors: lessons learned from early clinical trials. Nat Rev Clin Oncol 2013;10:143–53. doi:10.1038/nrclinonc.2013.10.

[9] Liu P, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. Nat Rev Drug Discov 2009;8:627–44. doi:10.1038/nrd2926.

[10] Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, et al. High frequency of mutations of the PIK3CA gene in human cancers. Science 2004;304:554. doi:10.1126/science.1096502.

[11] Koti M, Gooding RJ, Nuin P, Haslehurst A, Crane C, Weberpals J, et al. Identification of the IGF1/PI3K/NF κB/ERK gene signalling networks associated with chemotherapy resistance and treatment response in high-grade serous epithelial ovarian cancer. BMC Cancer 2013;13:549. doi:10.1186/1471-2407-13-549.

[12] Saura C, Bendell J, Jerusalem G, Su S, Ru Q, De Buck S, et al. Phase Ib study of Buparlisib plus Trastuzumab in patients with HER2-positive advanced or metastatic breast cancer that has progressed on Trastuzumab-based therapy. Clin Cancer Res 2014;20:1935–45. doi:10.1158/1078-0432.CCR-13-1070.

[13] Baselga J, Im S-A, Iwata H et al. PIK3CA status in circulating tumor DNA (ctDNA) predicts efficacy of buparlisib (BUP) plus fulvestrant (FULV) in postmenopausal women with endocrine-resistant HR+/HER2–advanced breast cancer (BC): first results from the randomized, phase III BELLE-2 trial. Present San Antonio Breast Cancer Symp Dec 8–12, 2015; San Antonio, TX, USA Abstr S6-01.

[14] Janku F, Hong DS, Fu S, Piha-Paul SA, Naing A, Falchook GS, et al. Assessing PIK3CA and PTEN in Early-Phase Trials with PI3K/AKT/mTOR Inhibitors. Cell Rep 2014;6:377–87. doi:10.1016/j.celrep.2013.12.035.

[15] EMA. European Medicines Agency - Find Medicine - Zydelig. 2016. http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/003843/human_med_001803.jsp&mid=WC0b01ac058001d124 (accessed March 10, 2016).

[16] FDA. Approved Drugs > Idelalisib. 2015 2016.

[17] van der Kuip H, Wohlbold L, Oetzel C, Schwab M, Aulitzky WE. Mechanisms of clinical resistance to small molecule tyrosine kinase inhibitors targeting oncogenic tyrosine kinases. Am J Pharmacogenomics 2005;5:101–12.

[18] Barouch-Bentov R, Sauer K. Mechanisms of drug resistance in kinases. Expert Opin Investig Drugs 2011;20:153–208. doi:10.1517/13543784.2011.546344.

[19] Daub H, Specht K, Ullrich A. Strategies to overcome resistance to targeted protein kinase inhibitors. Nat Rev Drug Discov 2004;3:1001–10. doi:10.1038/nrd1579.

[20] Meadows S, Rick S, Anella Y, Liu J, Li L, Yue P et al. Up-Regulation of the PI3K Signaling Pathway Mediates Resistance to Idelalisib. Blood 2015;126:3707.

[21] Elkabets M, Vora S, Juric D, Morse N, Mino-Kenudson M, Muranen T, et al. mTORC1

inhibition is required for sensitivity to PI3K p110α inhibitors in PIK3CA-mutant breast cancer. Sci Transl Med 2013;5:196ra99. doi:10.1126/scitranslmed.3005747.

[22] Beaufils F, Cmiljanovic N, Cmiljanovic V, Bohnacker T, Melone A, Marone R, et al. 5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-(trifluoromethyl)pyridin-2-amine (PQR309), a Potent, Brain-Penetrant, Orally Bioavailable, Pan-Class I PI3K/mTOR Inhibitor as Clinical Candidate in Oncology. J Med Chem 2017;60:7524–38. doi:10.1021/acs.jmedchem.7b00930.

[23] Tarantelli C, Gaudio E, Kwee I, Rinaldi A, Bernasconi E, Cascione L et al. Abstract 2652: Pre-clinical activity and mechanism of action of the novel dual PI3K/mTOR inhibitor PQR309 in B-cell lymphomas. Cancer Res 2015;752652.

[24] Cmiljanovic V, Cmiljanovic N, Marone R, Beaufils F, Zhang X, Zvelebil M et al. Abstract 2664: PQR309: Structure-based design, synthesis and biological evaluation of a novel, selective, dual pan-PI3K/mTOR inhibitor. Cancer Res 2015;752664.

[25] Cmiljanovic V, Ettlin RA, Beaufils F, Dieterle W, Hillmann P, Mestan J et al. Abstract 4514: PQR309: A potent, brain-penetrant, dual pan-PI3K/mTOR inhibitor with excellent oral bioavailability and tolerability. Cancer Res 2015;754514.

[26] Bohnacker T, Prota AE, Beaufils F, Burke JE, Melone A, Inglis AJ, et al. Deconvolution of Buparlisib's mechanism of action defines specific PI3K and tubulin inhibitors for therapeutic intervention. Nat Commun 2017;8:14683. doi:10.1038/ncomms14683.

[27] Tarantelli C, Gaudio E, Arribas AJ, Kwee I, Hillmann P, Rinaldi A, et al. PQR309 Is a Novel Dual PI3K/mTOR Inhibitor with Preclinical Antitumor Activity in Lymphomas as a Single Agent and in Combination Therapy. Clin Cancer Res 2017. doi:10.1158/1078-0432.CCR-17-1041.

[28] Di Leo A, Keun SL, Ciruelos E, Lønning P, Janni W, O'Regan R, et al. BELLE-3: A Phase III study of buparlisib + fulvestrant in postmenopausal women with HR+, HER2-, aromatase inhibitor-treated, locally advanced or metastatic breast cancer, who progressed on or after mTOR inhibitor-based treatment. San Antonio Breast Cancer Symp 2016;Abstract S4-07.

[29] Cheng H, Zou Y, Ross JS, Wang K, Liu X, Halmos B, et al. RICTOR Amplification Defines a Novel Subset of Patients with Lung Cancer Who May Benefit from Treatment with mTORC1/2 Inhibitors. Cancer Discov 2015;5:1262–70. doi:10.1158/2159-8290.CD-14-0971.

Figure and table legends

Table 1

Baseline demographics and clinical characteristics.

Table 2

Dose level, primary tumor, treatment duration, response, and genotype.

Table 3

Adverse events of grade 1-2 and grade 3 or worse.

Table 4

Analysis of PTB in 13 patients. The table indicates the exact day of the drug administration cycle the biopsy was taken, the delay after the last dose of oral PQR309, the 1mg dose-normalized Area Under the Curve from the beginning and until 24 hours (AUC_{0-24}), and the tumor content on the analysed tumor slices.

Figure 1

Activation of phosphorylation sites in the PI3K-mTOR signaling axis after 21 days of treatment with PQR309. The graphs on the left hand side show the change of the level of phosphorylation of a specific phosphorylation site while the patient is on therapy, in comparison to the baseline (first biopsy, equivalent to 100%). One data point represents 1 patient. The colour-code is explained in Tab. 4. The data-point corresponds to the mean of two independent measurements of the respective phospho-protein per biopsy and time-point. On the right hand side, patients were divided into one group, whose tumors grew despite therapy (round symbols), and a second group, whose tumors shrank (square symbols). Growth or shrinkage was defined by the best response of each patient. We considered all lesions that were also considered target lesions for the assessment according to RECIST 1.1.

Figure 2

Infiltration of the tumor with CD3, CD4, CD8 and FoxP3 positive immune cells (panels A-D, as indicated). Red dots = immune infiltates in patients with tumor growth. Green dots = immune infiltrates in patients with tumor shrinkage. Left-hand column: Quantification of immune infiltrates. Right-hand column: Exemplary image of IHC staining. Bar = 10 μm.

Table 1

Table 1. Baseline demographics and clinical characteristics

	Patients (n=28)
Sex	
Male	8
Female	20
Age (years)	58 (21-75)
ECOG performance status	
0	12
1	16
Tumor type	
Colorectal cancer	7
Ovarian cancer	6
Endomentrial carcinosarcoma	2
Lung cancer	2
Breast cancer	2
Other	9
Previous lines of systemic treatment	4 (0-9)
0	1
1	2
2	5
≥3	20

Table 2

Table 2. **Dose level, primary tumor, treatment duration, response, and genotype.**

Patient Number	Dose PQR309	Tumor type	Treatment duration (days)	DLT	Best response	Gene mutation	AA Change
6713_001	10 mg	Ewing-sarcoma	26	None	PD		
6713_002	15 mg	Colorectal carcinoma	25	None	PD		
6713_003	50 mg	Breast carcinoma	40	None	PD	TP53	p.Glu198Ter
6713_005	40 mg	Ovarian carcinoma	84	None	SD		
6713_006	40 mg	Ovarian carcinoma	7	NE	NE		
6713_007	30 mg	Colorectal carcinoma	41	None	PD		
6713_008	80 mg	Bartholin's gland carcinoma	152	None	SD	SMARCB1	p.Thr72Lys
6713_010	60 mg	Breast carcinoma	42	None	PD		
6713_011	60 mg	Ovarian carcinoma	42	None	SD		
6713_012	120 mg	Ovarian carcinoma	31	None	SD	PIK3CA JAK3	p.Ile391Met p.Pro132Thr
6713_013	120 mg	Cholangio carcinoma	8	NE	NE		
6713_014	120 mg	Colorectal carcinoma	42	None	PD		
6713_015	90 mg	Lung carcinoma	12	None	NE		
6713_018	150 mg	Lung carcinoma	31	None	SD		
6713_019	90 mg	Ovarian carcinoma	36	None	PD		
6713_021	80 mg	Mesothelioma	37	None	PD	MET	p.Arg988Cys
6713_022	80 mg	Colorectal carcinoma	40	None	PD	KRAS	p.Gly12Ser
6713_023	80 mg	Endometrial carcinoma	46	None	SD		

6713_024	100 mg	Squamous cell cancer of the tongue	44	None	PD	NOTCH1 ATM	p.Pro2465Leu p.Ser1691Arg
6713_026	100 mg	Colorectal carcinoma	83	None	SD	KRAS APC TP53 TP53	p.Gln61His p.Ser1315* p.Ser240Cys p. Gly244Ser
6713_028	100 mg	Endometrial carcinoma	42	None	PD		
6713_029	100 mg	Thymic carcinoma	446	None	PR	RICTOR1 amplification	
6713_031	100 mg	Colorectal carcinoma	20	G3 Rash G4 Suicide attempt	PD	TP53 PTEN	p.Ile255Thr p.Gln110Ter
6713_032	100 mg	Sinonasal carcinoma	55	None	SD	PIK3CA	p.Glu545Lys
6713_033	100 mg	Ovarian carcinoma	50	G3 Fatigue G3 Diarrhea G3 Transaminitis	SD		
6713_034	80 mg	Colorectal carcinoma	18	G3 Hyperglycemia	NE		
6713_035	80 mg	Endometrial carcinoma	21	None	PD		
6713_036	80 mg	Cervical carcinoma	63	None	PD	KIT	p.Met541Leu

DLT=dose limiting toxicity. PD=progressive disease. SD=stable disease. PR=partial response. CR=complete response. NE=not evaluable. Tumor assessment: clinically every 3 weeks, by CT or MRI every 6 weeks for the first 4 cycles. Thereafter every 9 weeks.

* = non-sense substitution.

Table 3

Table 3. **Adverse Events of Grade 1-2 and Grade 3 or worse**

	NCI CTC severity grade		Total
	1-2	3-4	
Patients with at least one AE	28 (100%)	21 (75.0%)	28 (100%)
Fatigue	23 (82.1%)	3 (10.7%)	26 (92.9%)
Hyperglycaemia	17 (60.7%)	7 (25.0%)	24 (85.7%)
Decreased appetite	15 (53.6%)	0	15 (53.6%)
Diarrhoea	12 (42.9%)	3 (10.7%)	15 (53.6%)
Nausea	14 (50.0%)	1 (3.6%)	15 (53.6%)
Rash maculo-papular	8 (28.6%)	5 (17.9%)	13 (46.4%)
Weight decreased	13 (46.4%)	0	13 (46.4%)
Vomiting	11 (39.3%)	1 (3.6%)	12 (42.9%)
Abdominal pain	10 (35.7%)	0	10 (35.7%)
Constipation	9 (32.1%)	0	9 (32.1%)
Hypertension	5 (17.9%)	4 (14.3%)	9 (32.1%)
Pruritus	8 (28.6%)	0	8 (28.6%)
Depression	6 (21.4%)	0	6 (21.4%)
Dyspnoea	6 (21.4%)	0	6 (21.4%)
Dry skin	5 (17.9%)	0	5 (17.9%)
Alanine aminotransferase increased	2 (7.1%)	2 (7.1%)	4 (14.3%)
Cough	4 (14.3%)	0	4 (14.3%)
Oedema peripheral	4 (14.3%)	0	4 (14.3%)
Urinary tract infection	4 (14.3%)	0	4 (14.3%)
Agitation	3 (10.7%)	0	3 (10.7%)
Aspartate aminotransferase increased	1 (3.6%)	2 (7.1%)	3 (10.7%)
Back pain	2 (7.1%)	1 (3.6%)	3 (10.7%)
Disease progression	0	3 (10.7%)	3 (10.7%)
Insomnia	3 (10.7%)	0	3 (10.7%)
Pollakiuria	3 (10.7%)	0	3 (10.7%)
Stomatitis	3 (10.7%)	0	3 (10.7%)
Abdominal pain upper	2 (7.1%)	0	2 (7.1%)
Alopecia	2 (7.1%)	0	2 (7.1%)
Ascites	1 (3.6%)	1 (3.6%)	2 (7.1%)
Cognitive disorder	2 (7.1%)	0	2 (7.1%)
Dizziness	2 (7.1%)	0	2 (7.1%)
Embolism	2 (7.1%)	0	2 (7.1%)
Gamma-glutamyltransferase increased	1 (3.6%)	1 (3.6%)	2 (7.1%)
Haematuria	2 (7.1%)	0	2 (7.1%)
Headache	1 (3.6%)	1 (3.6%)	2 (7.1%)
Hyponatraemia	0	2 (7.1%)	2 (7.1%)
Insulin C-peptide increased	2 (7.1%)	0	2 (7.1%)

Table 3. **Adverse Events of Grade 1-2 and Grade 3 or worse**

	NCI CTC severity grade		Total
	1-2	3-4	
Muscular weakness	2 (7.1%)	0	2 (7.1%)
Pain	2 (7.1%)	0	2 (7.1%)
Palmar-plantar erythrodysaesthesia syndrome	2 (7.1%)	0	2 (7.1%)
Polydipsia	2 (7.1%)	0	2 (7.1%)
Pyrexia	2 (7.1%)	0	2 (7.1%)
Rash	2 (7.1%)	0	2 (7.1%)
Seizure	2 (7.1%)	0	2 (7.1%)
Abdominal discomfort	1 (3.6%)	0	1 (3.6%)
Abdominal distension	1 (3.6%)	0	1 (3.6%)
Amnesia	1 (3.6%)	0	1 (3.6%)
Anxiety	1 (3.6%)	0	1 (3.6%)
Asthenia	1 (3.6%)	0	1 (3.6%)
Blood alkaline phosphatase increased	1 (3.6%)	0	1 (3.6%)
Blood bilirubin increased	1 (3.6%)	0	1 (3.6%)
Blood cholesterol increased	1 (3.6%)	0	1 (3.6%)
Blood creatine phosphokinase increased	1 (3.6%)	0	1 (3.6%)
Bone pain	1 (3.6%)	0	1 (3.6%)
Bronchitis	1 (3.6%)	0	1 (3.6%)
Bronchopneumonia	0	1 (3.6%)	1 (3.6%)
Confusional state	1 (3.6%)	0	1 (3.6%)
Deafness	1 (3.6%)	0	1 (3.6%)
Dermatitis acneiform	1 (3.6%)	0	1 (3.6%)
Disturbance in attention	1 (3.6%)	0	1 (3.6%)
Dry eye	1 (3.6%)	0	1 (3.6%)
Dry mouth	1 (3.6%)	0	1 (3.6%)
Dysgeusia	1 (3.6%)	0	1 (3.6%)
Dyspepsia	1 (3.6%)	0	1 (3.6%)
Dysphagia	1 (3.6%)	0	1 (3.6%)
Dysphonia	1 (3.6%)	0	1 (3.6%)
Enteritis	1 (3.6%)	0	1 (3.6%)
Flank pain	1 (3.6%)	0	1 (3.6%)
Gastritis	1 (3.6%)	0	1 (3.6%)
Gastrooesophageal reflux disease	1 (3.6%)	0	1 (3.6%)
Glossitis	1 (3.6%)	0	1 (3.6%)
Hepatic pain	1 (3.6%)	0	1 (3.6%)
Hypersensitivity	1 (3.6%)	0	1 (3.6%)
Hypokalaemia	1 (3.6%)	0	1 (3.6%)
Hypomagnesaemia	1 (3.6%)	0	1 (3.6%)
Hypotension	1 (3.6%)	0	1 (3.6%)

Table 3. **Adverse Events of Grade 1-2 and Grade 3 or worse**

	NCI CTC severity grade		Total
	1-2	3-4	
Hypothermia	1 (3.6%)	0	1 (3.6%)
Hypoxia	0	1 (3.6%)	1 (3.6%)
Insulin C-peptide	1 (3.6%)	0	1 (3.6%)
Irritability	1 (3.6%)	0	1 (3.6%)
Lethargy	1 (3.6%)	0	1 (3.6%)
Lower respiratory tract infection	0	1 (3.6%)	1 (3.6%)
Lung infection	0	1 (3.6%)	1 (3.6%)
Lymph node pain	1 (3.6%)	0	1 (3.6%)
Mucosal infection	1 (3.6%)	0	1 (3.6%)
Muscle spasms	1 (3.6%)	0	1 (3.6%)
Neck injury	1 (3.6%)	0	1 (3.6%)
Non-cardiac chest pain	1 (3.6%)	0	1 (3.6%)
Oesophageal pain	1 (3.6%)	0	1 (3.6%)
Pain in extremity	1 (3.6%)	0	1 (3.6%)
Photopsia	1 (3.6%)	0	1 (3.6%)
Pleural effusion	1 (3.6%)	0	1 (3.6%)
Polyuria	1 (3.6%)	0	1 (3.6%)
Post procedural haematuria	0	1 (3.6%)	1 (3.6%)
Proctalgia	1 (3.6%)	0	1 (3.6%)
Psychotic disorder	0	1 (3.6%)	1 (3.6%)
Suicide attempt	0	1 (3.6%)	1 (3.6%)
Toothache	1 (3.6%)	0	1 (3.6%)
Tremor	1 (3.6%)	0	1 (3.6%)
Tumour pain	0	1 (3.6%)	1 (3.6%)
Upper respiratory tract infection	1 (3.6%)	0	1 (3.6%)
Urinary tract pain	1 (3.6%)	0	1 (3.6%)
Vaginal infection	1 (3.6%)	0	1 (3.6%)
Vertigo	1 (3.6%)	0	1 (3.6%)
Visual acuity reduced	1 (3.6%)	0	1 (3.6%)
Vulvovaginal dryness	1 (3.6%)	0	1 (3.6%)
Vulvovaginal pain	1 (3.6%)	0	1 (3.6%)

A patient with multiple occurrences of an AE is counted only once (with the worst grade) in the AE category.

Table 4

Table 4

#	UPN	Cycle Day	Hours after last PQR309 intake	Dose-normalized AUC ((h*ng/mL)/mg)	Overall response	Target lesion sum - change from baseline [%]	Symbol
1	003	C2D5	2	58.2	**PD**	-8.7%	
2	008	C1D16	23	6.95	SD	-15.0%	
3	012	C2D1	24	80.7	SD	-7.3%	
4	014	C2D1	22	126	**PD**	**18.8%**	
5	021	C2D7	3	93.4	**PD**	-61.3%	
6	022	C1D19	22	15.5	**PD**	**17.5%**	
7	026	C2D5	5	80.9	SD	-9.2%	
8	028	C1D18	29	65.3	**PD**	**2.1%**	
9	031	C2D1	27	65.2	**PD**	**8.6%**	
10	032	C2D7	26	67.6	SD	-25.0%	
11	033	C2D4	4	45.7	SD	**2.7%**	
12	035	C1D21	2	64.2	**PD**	-11.9%	
13	036	C2D1	25	120	**PD**	22.0%	

Figure 1

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Fig. 1

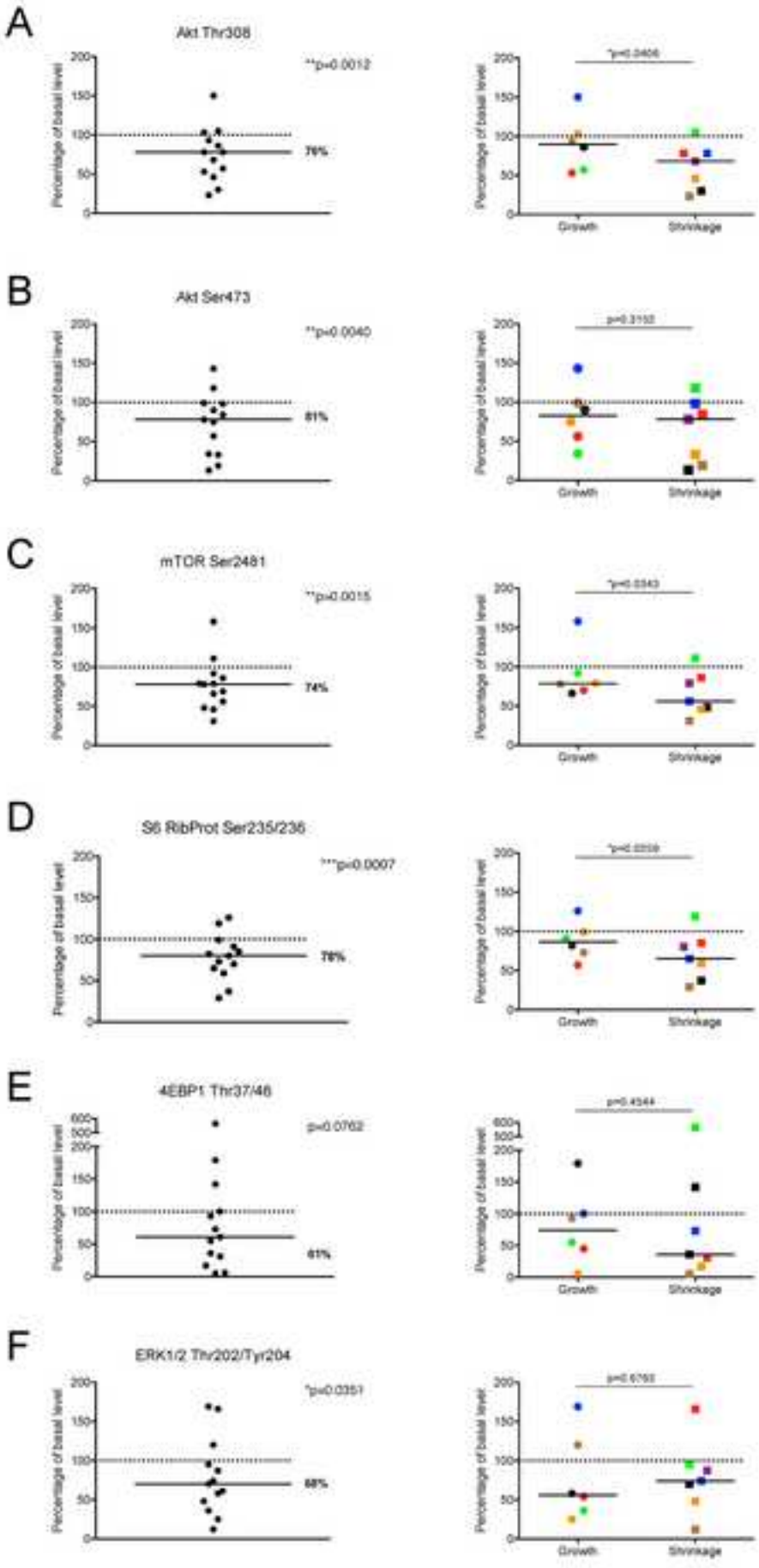

Figure 2
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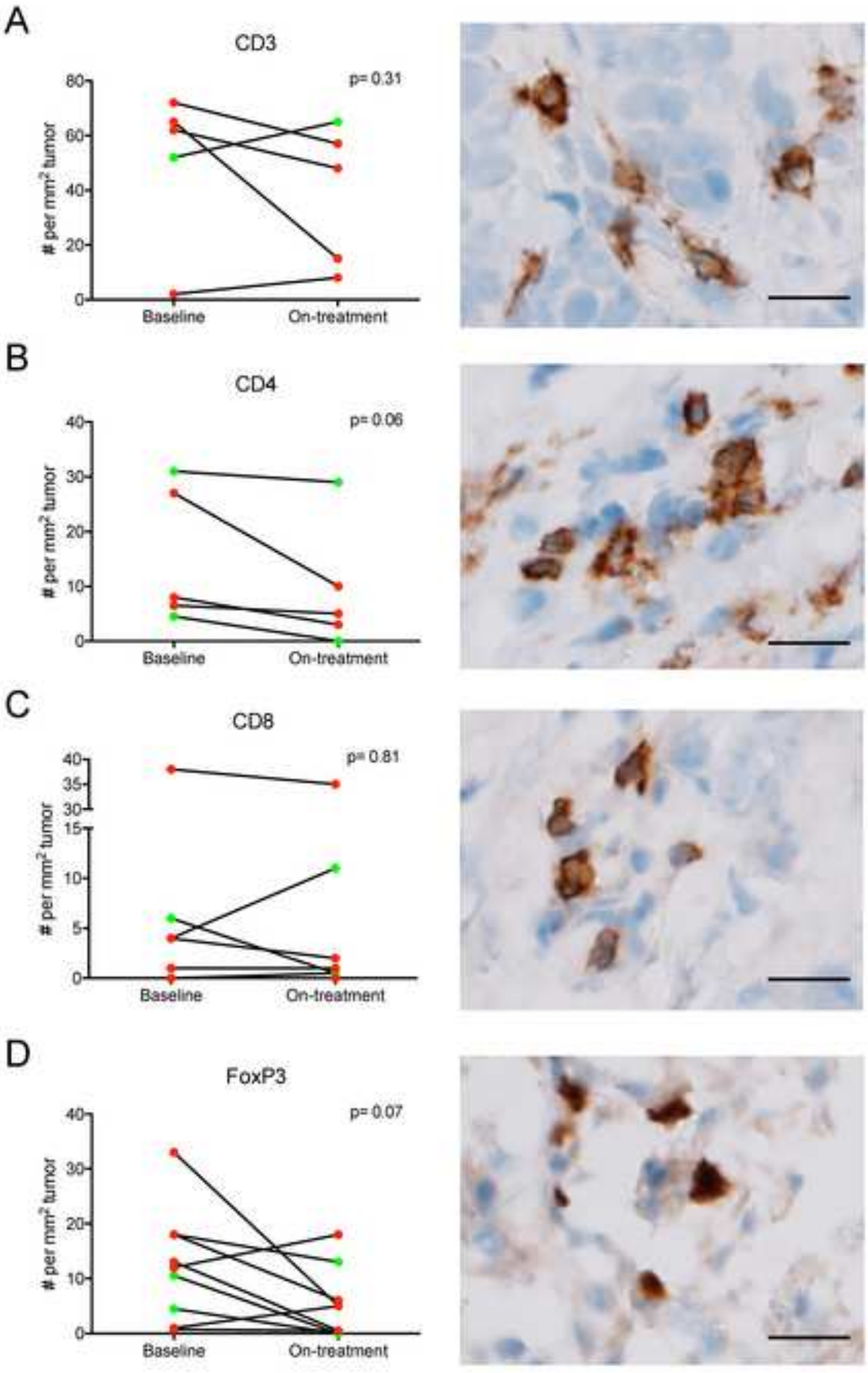
Fig. 2



Conflicts of interest

NC, VC, MS, SD and RH: Employees of Piqur.
The other authors declare no conflict of interest.

Supplementary Text

Click here to download Supplementary Text or Table (online publication only): PQR309-001_Manuscript_suppl_EJCA.docx

Supplementary Table 1

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Supplementary Figure 1

Suppl. Figure 1

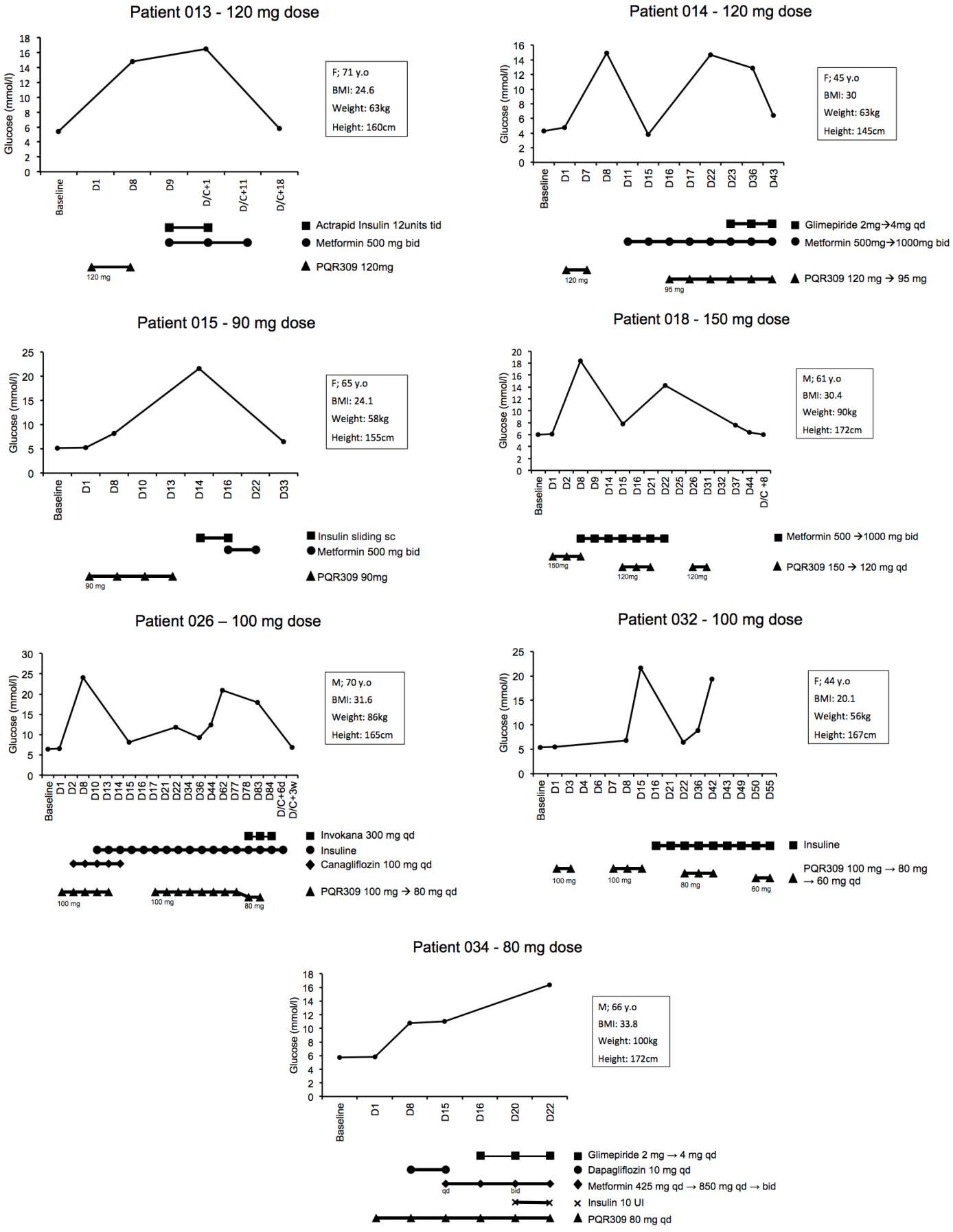
#	Symbol	GeneBank	#	Symbol	GeneBank
1	ADAR	NM_001111	43	MAPK3	NM_002746
2	AKT1	NM_005163	44	MAPK8	NM_002750
3	AKT2	NM_001626	45	MTCP1	NM_001018025
4	AKT3	NM_005465	46	MTOR	NM_004958
5	APC	NM_000038	47	MYD88	NM_002468
6	BAD	NM_004322	48	NFKB1	NM_003998
7	BTK	NM_000061	49	NFKBIA	NM_020529
8	CASP9	NM_001229	50	PABPC1	NM_002568
9	CCND1	NM_053056	51	PAK1	NM_002576
10	CD14	NM_000591	52	PDGFRA	NM_006206
11	CDC42	NM_001791	53	PDK1	NM_002610
12	CDKN1B	NM_004064	54	PDK2	NM_002611
13	CHUK	NM_001278	55	PDPK1	NM_002613
14	CSNK2A1	NM_001895	56	PIK3CA	NM_006218
15	CTNNB1	NM_001904	57	PIK3CG	NM_002649
16	EIF2AK2	NM_002759	58	PIK3R1	NM_181504
17	EIF4B	NM_001417	59	PIK3R2	NM_005027
18	EIF4E	NM_001968	60	PRKCA	NM_002737
19	EIF4EBP1	NM_004095	61	PRKCB	NM_002738
20	EIF4G1	NM_182917	62	PRKCZ	NM_002744
21	ELK1	NM_005229	63	PTEN	NM_000314
22	FASLG	NM_000639	64	PTK2	NM_005607
23	FKBP1A	NM_000801	65	PTPN11	NM_002834
24	FOS	NM_005252	66	RAC1	NM_006908
25	FOXO1	NM_002015	67	RAF1	NM_002880
26	FOXO3	NM_001455	68	RASA1	NM_002890
27	GJA1	NM_000165	69	RBL2	NM_005611
28	GRB10	NM_005311	70	RHEB	NM_005614
29	GRB2	NM_002086	71	RHOA	NM_001664
30	GSK3B	NM_002093	72	RPS6KA1	NM_002953
31	HRAS	NM_005343	73	RPS6KB1	NM_003161
32	HSPB1	NM_001540	74	SHC1	NM_003029
33	IGF1	NM_000618	75	SOS1	NM_005633
34	IGF1R	NM_000875	76	SRF	NM_003131
35	ILK	NM_004517	77	TCL1A	NM_021966
36	IRAK1	NM_001569	78	TIRAP	NM_001039661
37	IRS1	NM_005544	79	TLR4	NM_138554
38	ITGB1	NM_002211	80	TOLLIP	NM_019009
39	JUN	NM_002228	81	TSC1	NM_000368
40	MAP2K1	NM_002755	82	TSC2	NM_000548
41	MAPK1	NM_002745	83	WASL	NM_003941
42	MAPK14	NM_001315	84	YWHAH	NM_003405

Supplementary Figure 1

List of 88 PI3K-mTOR associated mRNAs analysed in PTB.

Supplementary Figure 2

Suppl. Figure 2

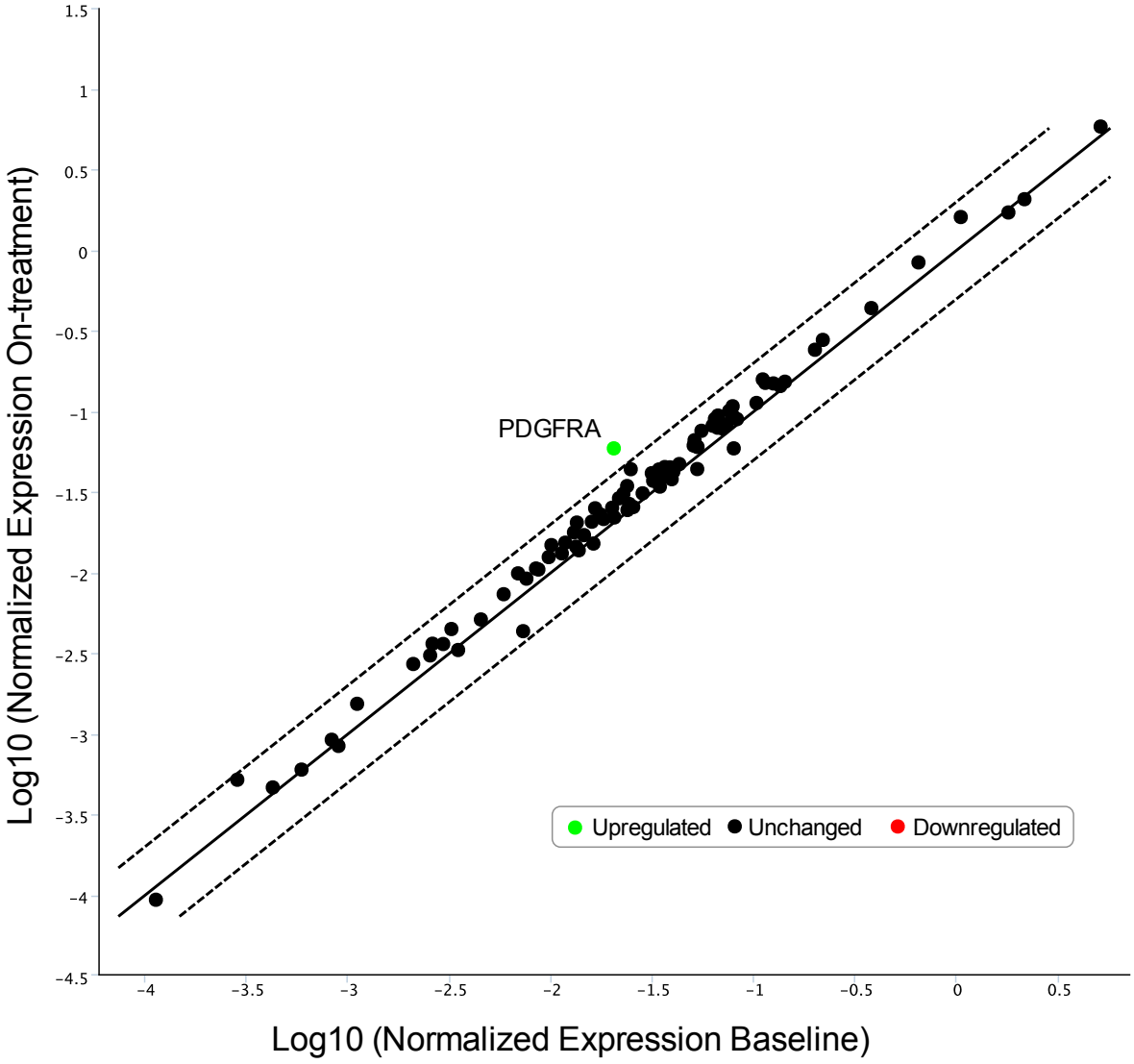


Supplementary Figure 2

Glucose levels and treatment of hyperglycemia in patients with hyperglycemia grade 3-4. Individual data are presented for all 7 patients with hyperglycemia grade ≥3.

Supplementary Figure 3

Suppl. Figure 3

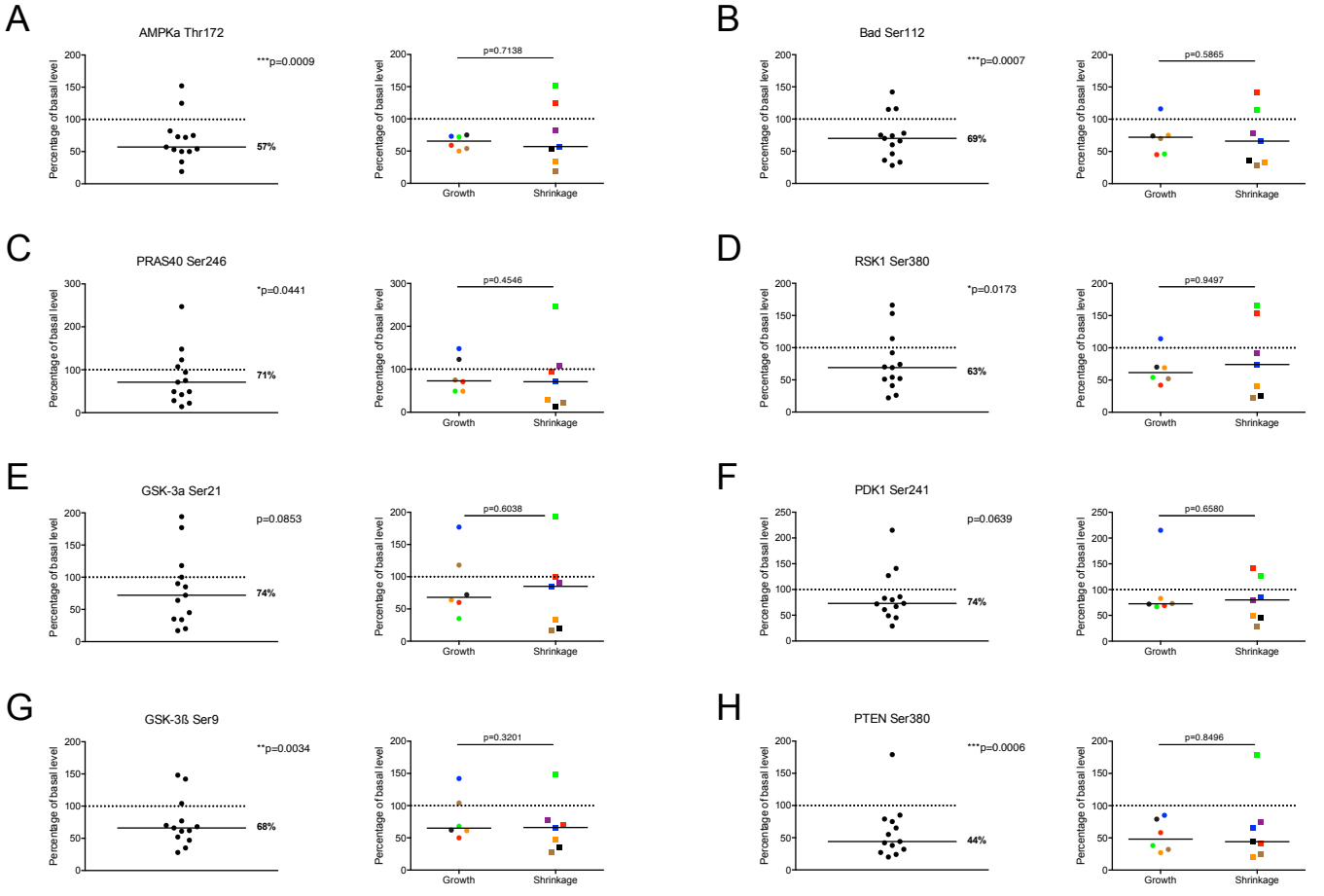


Supplementary Figure 3

Changes of the expression level of 88 mRNAs associated with PI3K-mTOR signaling after 21 days of treatment with PQR309. PDGFRA is the only mRNA showing a trend towards upregulation. No statistically significant up- or down-regulation was observed.

Supplementary Figure 4

Suppl. Figure 4



Supplementary Figure 4

Full panel of phospho-sites analysed in addition to those presented in Fig. 1. The colour-code is explained in Tab. 4.