Supplementary material

Supplementary Table 1

Sample	$T_{1\rho}$ (1)	$T_{1\rho}$ (2)	$T_{1\rho}$ (3)	wLOGSY
BKM120	0.88	0.85	0.70	-43.7
BKM120 / tubulin (50:1)	0.76	0.72	0.52	43.4
BKM120 / tubulin (20:1)	0.44	0.35	0.15	87.4

Note: Ratio of signal intensity of the three aromatic resonances of BKM120 $T_{1\rho}$ (200ms) / $T_{1\rho}$ (10ms) and signal intensity measured by wLOGSY as function of tubulin concentration (see Figure 5C).

Material and Methods

Material and antibodies: Anti-HA (#9110) and anti-Akt (# 05-591) antibodies were from Abcam and Upstate, respectively.

In vivo efficacy studies: All experimental procedures of the efficacy studies were approved by the Kantonales Veterinäramt Basel-Stadt under license #1769. Antitumor activity is expressed as T/C % (mean increase of tumor volumes of treated animals divided by the mean increase of tumor volumes of control animals, multiplied by 100). Data are presented as means ± one standard error of the mean (SEM). Comparisons between groups and vehicle control group were done using either one-way ANOVA or ANOVA on ranks followed by Dunnett's tests when data were, respectively, either normally distributed or not. For all tests, the level of

significance was set at $p<0.05$. Calculations were performed using SigmaStat v 2.03 (Jandel Scientific).

Supplementary figure legend

Sup Figure 1: Sensitivity profiles of GDC-0941 and BKM120 in a panel of 381 cell lines. The data are scaled by the positive control (1μM MG132) and the negative control (DMSO). The percentage of maximum activity (Amax) is represented in function of the crossing point (concentration in μM at 50% of MG132 activity). Each data point represents a cell line; the vertical and horizontal lines represent, respectively, the median of the crossing point values (1.33μM) and the median of the Amax values (-90.06%), of BKM120 across all cell lines. The population of cell lines least responding to GDC-0941, among which some are sensitive to BKM120, are highlighted in green.

Sup Figure 2: Characterization of the MCF7 cells stably expressing a dominant active form of Akt. A. Upon infection with viral particles obtained from the pBabe-puro-myr-Akt retroviral vectors, pools were selected upon addition of puromycin and maintained in culture for several weeks. Extracts from pools were generated for the indicated time of culture and analyzed by Western-blot for the expression of the exogenously expressed HA-tagged form of the dominant active myr-Akt protein. B. $2.5x10^6$ MCF7-BP (upper panel) or MCF7-myr-Akt (bottom panel) were seeded in 10 cm dishes and exposed for 1 h to the indicated compounds at the indicated concentrations (in nM). Cells were then lyzed and cell extracts were analyzed by Western-blot for pathway inhibition as revealed by S473P-Akt levels.

Sup Figure 3: Effects of GDC-0941 and BEZ235 on the cell cycle. A and B. $2x10^6$ A2058 cells were seeded in 10 cm dishes and incubated for 24 h with 5 μM of either GDC-0941 (A) or BEZ235 (B). Cells were then fixed, prepared as described for quantification of the population in the different phases of the cell cycle by fluorescence-activated cell sorting. G1, S and G2/M distribution for control untreated cells are described in the mean text and in Figure 4A.

Sup Figure 4: BKM120 provokes a prometaphase to metaphase block. A and B. MDA-MB231 (A) and U87MG (B) cells grown on coverslips were treated for 24 h either with BKM120 (5 μM) or Nocodazole (100 nM) and the effects of compound treatment on microtubule dynamics and G2/M arrest was monitored by immuno-fluorescence staining of alpha-tubulin (microtubules), gamma tubulin (centrosomes) and DAPI (DNA). Pictures were taken with a 100X objective.

Sup Figure 5: A. Effects of Paclitaxel and Nocodazole on Tubulin polymerization. Tubulin was mixed with either Paclitaxel (10 μM), Nocodazole (10 μM) or the DMSO control in the presence of GTP. The polymerization of monomeric tubulin into microtubule was started by transferring the reaction tubes from 4°C to 37°C, and monitored by the increase in absorbance (λ=340 nM) over a period of 60 min. B. Competition experiments of NVP-BKM120 with colchicine and podophyllotoxin by NMR spectroscopy. $T_{1\rho}$ relaxation of BKM120 in the presence of tubulin (50-fold excess of compound) remains unchanged after adding

podophyllotoxin or colchicine, as emphasized by the drawn arrows. The spectra of the three compounds are shown in three colors at the bottom.

Sup Figure 6: Effects of BKM120 exposure on pHistone H3 levels and U87MG tumor growth. A. $2.5x10^6$ Rat1-myr-p110a (left) or U87MG cells were seeded in 10 cm dishes and exposed for 6h either to BKM120 (5 μM) or to the DMSO control. Cells were then lyzed and cell extracts were analyzed by Western-blot for PI3K pathway inhibition and G2/M markers as revealed by S473P-Akt (upper panel) and Ser10P-Histone H3 (bottom panel) levels, respectively. B. U87MG tumor bearing mice were treated orally, once per day for 6 days with BKM120 at the indicated doses. Tumor volumes were callipered and plotted. ns: not significant.

Sup Figure 7: BKM120 induced prometaphase block could be reverted. A2058 (upper panels), MDA-MB231 (lower panels) and U87MG cells grown on coverslips were treated for 6 h, 6 h with a subsequent 18 h washout period or 24 h with either 5 μM BKM120 or 100 nM Nocodazole and the effects of compound treatment on microtubule dynamics and G2/M arrest was monitored by immuno-fluorescence staining of alpha-tubulin. Pictures were taken with a 40X objective.