

Serveur Académique Lausannois **SERVAL** serval.unil.ch

Author Manuscript

Faculty of Biology and Medicine Publication

This paper has been peer-reviewed but does not include the final publisher proof-corrections or journal pagination.

Published in final edited form as:

Title: Rebound pathway overactivation by cancer cells following discontinuation of PI3K or mTOR inhibition promotes cancer cell growth.

Authors: Faes S, Santoro T, Troquier L, De Souza Silva O, Dormond O

Journal: Biochemical and biophysical research communications

Year: 2019 Jun 4

Issue: 513

Volume: 3

Pages: 546-552

DOI: 10.1016/j.bbrc.2019.04.044

Rebound pathway overactivation by cancer cells following discontinuation of PI3K or mTOR inhibition promotes cancer cell growth

Seraina Faes ^a, Tania Santoro ^{a, 1}, Laetitia Troquier ^a, Olga De Souza Silva ^a, Olivier Dormond ^{a, *}

^a Department of Visceral Surgery, Lausanne University Hospital, University of Lausanne, Pavillon 4, Av de Beaumont, 1011 Lausanne, Switzerland

¹ Present address: Department of Medicine, Lausanne University Hospital, University of Lausanne, Rue du Bugnon 46, 1011 Lausanne, Switzerland

* Corresponding Author: Department of Visceral Surgery, Lausanne University Hospital, University of Lausanne, Pavillon 4, Av de Beaumont, 1011 Lausanne, Switzerland. *E-mail address*: olivier.dormond@chuv.ch tel: ++41 79 556 03 40

seraina.faes@chuv.ch, tania.santoro@chuv.ch, laetitia.troquier@chuv.ch, olga.de-souza-silva@chuv.ch, olivier.dormond@chuv.ch

Summary

Whilst effects of anti-cancer drugs have been thoroughly explored, little is known about the repercussion of drug cessation. However, this has important clinical relevance since several clinical protocols such as intermittent drug scheduling lead to frequent drug discontinuation. In this study, we have thus investigated the consequences of withdrawal of agents that target the PI3K/AKT/mTOR signaling pathway in cancer cells. We report that washout of kinase inhibitors of mTOR or PI3K inhibitors led to a rapid and sustainable overactivation of AKT. Consequently, proliferation of tumor cells was significantly higher following drug washout in cancer cells that were pre-treated with mTOR or PI3K inhibitors compared to untreated cells. This effect was prevented by the addition of an AKT inhibitor following drug washout. Rebound AKT overactivation induced by mTOR or PI3K inhibitors discontinuation was mediated by IGF-1R, as demonstrated by its prevention in the presence of an IGF-1R inhibitor and by increased IGF-1R phosphorylation in treated cells versus control cells. Taken together, our results show that discontinuation of PI3K or mTOR inhibitors results in AKT overactivation that promotes tumor growth. They further highlight the benefit of adding an AKT inhibitor following cessation of PI3K or mTOR inhibitors.

Key words: targeted therapies; mTOR; PI3K; washout; cancer.

1. Introduction

Blocking activated proteins that partake in cancer progression represents a treatment modality in cancer therapy [1]. In this context, the inhibition of the PI3K/AKT/mTOR signaling pathway represents a promising approach as it contributes to tumor progression and its components are frequently overactivated in cancer cells [2-4]. Accordingly, numerous chemical inhibitors targeting different elements of the pathway have been developed [5]. However, despite promising pre-clinical observations, their anti-cancer benefits in patients are limited. In fact, the inhibition of the PI3K/AKT/mTOR pathway is associated with several drawbacks. For instance, inhibition of PI3K or AKT upregulates the expression and activity of multiple receptor tyrosine kinases that attenuate the anti-cancer efficacy of PI3K or AKT inhibitors [6-8]. Similarly, inhibition of mTORC1 abrogates negative feedback loops resulting in the activation of ERK and AKT [9, 10]. Therefore, the cellular consequences of the inhibition of the PI3K/AKT/mTOR signaling pathway are complex and need to be explicitly identified in order to design adequate therapeutic strategies [11].

In the field of cancer therapy, most studies have analyzed the effects of targeted therapies in the presence of specific inhibitors. In contrast, little is known about the repercussion of drug withdrawal. This aspect has however important clinical consequences as several protocols are exploring intermittent drug scheduling, in part to avoid substantial toxicities generated by such therapies [12-15]. In addition, the use of oral anti-cancer drugs is increasing, resulting in self-administered treatments [16]. Hence, decreased therapy adherence could contribute to drug cessation. This represents a particular issue as adherence rates of less than 20% have been reported in cancer patients [17].

In this study, we have investigated the consequences of withdrawal of mTOR or PI3K inhibitors in cancer cells. We report that drug washout resulted in a rapid overactivation of AKT promoting cancer cell growth.

2. Materials and methods

2.1. Reagents and antibodies

PP242 (#CD0258), Ku-0063794 (#CD0274), and PX-866 (#CD0279) were from Chemdea (Ridgewood, NJ, USA). BKM-120 (#S2247), BYL719 (#S2814), MK-2206 (#S1078), NVP-AEW541 (#S1034), sapitinib (#S2192), and erlotinib (#S7786) were from Selleck Chemicals (Houston, TX, USA). Anti-phospho-AKT (#4060), anti-AKT (#2920), and anti phospho-IGF-1R β (#3024) antibodies were from Cell Signaling Technology (Danvers, MA, USA). Anti- β -Actin (#A2228) antibody was from Sigma-Aldrich (Buchs, Switzerland).

2.2. Cell culture

SW480, HT-29, LS174T, 786-O, and Huh-7 cells were cultured in Dulbecco's Modified Eagle's Medium-high glucose (DMEM) (Sigma-Aldrich, Buchs, Switzerland) supplemented with 10% FBS and 1% streptomycin/penicillin in a 5% CO₂ atmosphere and at 37°C.

2.2. MTS proliferation assay

Cancer cells were plated in 96 well plates at 10 000 cells per well. Twenty-four hours later cells were treated with PP242 (5 μ M) or BKM-120 (500nM) or DMSO as a control for six hours. Drugs were washed out by rinsing cells four times with PBS and cells were subsequently incubated for 48 hours in DMEM/10% FBS. For some experiments, cancer cells were treated with MK-2206 (1 μ M) or NVP-AEW541 (1 μ M) after drug washout. Cellular proliferation was monitored after 48 hours with CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS) (Promega, Madison, WI, USA) by following the manufacturer's instructions. Absorbance at 492nm was

measured 30 minutes after compound addition and expressed as a relative percentage compared to untreated control cells. Experiments were performed in triplicates and repeated three times.

2.3. Cell counting

Two hundred thousand cancer cells were plated in six well plates. Twenty-four hours later cells were treated with PP242 (5 μ M) or BKM-120 (500nM) or DMSO as a control for six hours. Drugs were washed out by rinsing cells four times with PBS and cells were subsequently incubated for 48 hours in DMEM/10% FBS. Subsequently, adherent cells were collected and trypan-blue negative cells were counted using a Neubauer hemocytometer. Results are expressed as relative mean cell count of three independent experiments compared to untreated control cells.

2.4 Western blot analysis

Two hundred thousand cancer cells were grown in six well plates and treated as indicated. Cells were washed twice with ice-cold PBS and lysed in RIPA buffer containing sodium orthovanadate and protease inhibitor cocktail (Santa Cruz Biotechnologies, Dallas, TX, USA). Protein concentrations were evaluated in lysates using BCA assay (Thermo Fischer Scientific, Waltham, MA, USA). Twenty micrograms of proteins per condition were separated on 4-12% polyacrylamide gels (Invitrogen, Waltham, MA, USA) and transferred to a polyvinylidene difluoride membrane. Odyssey blocking buffer (LI-COR Biosciences, Lincoln, NE, USA) was used to block membranes and, following incubation with primary and infrared secondary antibodies, bands from immunoreactive proteins were visualized by an Odyssey infrared imaging system. For some experiments densitometric analysis was performed using ImageJ software. Density values of phosphorylated proteins were normalized to total protein

for each sample. Unstimulated cells were given a value of 1.0, and ratios in all other samples were normalized to this value.

2.5. Statistical analysis

Student's t-tests were performed using GraphPad Prism version 8. $p < 0.05$ was considered statistically significant.

3. Results

3.1. *mTOR inhibitors or PI3K inhibitors washout in cancer cells increases AKT activity*

Whereas the effect of ATP-competitive inhibitors of mTOR on cancer cells has been thoroughly explored, little is known about the cellular consequences of drug cessation. To address this, SW480 or HT-29 colon cancer cells were treated with 5 μ M of PP242 [18] or 5 μ M of Ku-0063794 [19] for 24 hours followed by a drug washout of one hour. As expected, PP242 and Ku-0063794 inhibited mTORC2 activity as assessed by a decreased AKT phosphorylation of the serine 473 site [20]. Following one hour of drug washout, AKT (Ser 473) phosphorylation was however increased in cancer cells that were initially treated with PP242 or Ku-0063794 (Fig. 1A). Increased AKT phosphorylation following washout of mTOR inhibitors was not restricted to SW480 and HT-29 cancer cells as similar findings were obtained in Huh-7 hepatocellular carcinoma cells and 786-O renal carcinoma cancer cells (Fig. 1B). The washout effect was also long lasting as we could still observe increased AKT phosphorylation 48 hours after drug withdrawal (Fig. 1C). Short-term exposure to mTOR inhibitor was sufficient to increase AKT phosphorylation upon drug washout as this effect was already observed after 6 hours of pre-treatment (Fig. 1D). Finally, a similar rebound activation of AKT was observed upon drug withdrawal of PI3K

inhibitors. Treatment of cancer cells with PX-866 (500nM) [21] and BKM-120 (500nM) [22], both pan class I PI3K inhibitors as well as BYL719 (10 μ M) [23], a selective PI3K alpha inhibitor, resulted in reduced AKT phosphorylation (Fig. 1E). However, following one hour of drug washout, AKT phosphorylation was increased in cancer cells previously treated with PI3K inhibitors. Taken together, these results show that withdrawal of PI3K or mTOR inhibitors leads to increased activation of AKT.

3.2. mTOR or PI3K inhibitors washout promotes cancer cell growth

Since AKT promotes cell proliferation and survival, we next hypothesized that increased AKT activity following drug washout would increase cell proliferation. To test this SW480, HT-29, 786-O, and Huh-7 were treated with PP242 or BKM-120 for six hours. Drugs were washed out and cell proliferation was assessed 48 hours later using MTS proliferation assay or cell counting (Fig. 2A). We found that washout of PP-242 increased cell proliferation by 31.5%, 34.6%, 30.7%, and 32.6% in SW480, HT-29, 786-O, and Huh-7 respectively compared to DMSO treated cells (Fig. 2B). Cell proliferation was upregulated by 40.9% in SW480 cells, 39.3% in HT-29 cells, 39.5% in 786-O cells, and 38.3% in Huh-7 cells following BKM-120 discontinuation compared to DMSO treated cancer cells (Fig. 2B). Similar results were obtained by cell counting (Fig. 2C). These results suggest that interruption of PI3K or mTOR inhibitors promotes cancer cell proliferation.

3.3. AKT inhibitor MK-2206 blocks drug washout-mediated cancer cell growth

To address the role of AKT in increasing cancer cell growth following withdrawal of PP242 or BKM-120, cancer cells were treated with AKT inhibitor MK-2206 [24] following drug washout, and cell proliferation was monitored after 48 hours. We observed that as in Fig. 2 discontinuation of PP242 or BKM-120 increased cell

proliferation compared to control cells treated with DMSO. We further found that in presence of MK-2206 proliferation of DMSO control cells was reduced by 46% in SW480 and 786-O cells, by 38% in HT-29 cells and by 47% in Huh-7 cells (Fig. 3). In addition, MK-2206 prevented upregulation of cell proliferation induced by cessation of PP242 or BKM120. This suggests that overactivation of AKT following PP242 or BKM-120 discontinuation promotes cancer cell proliferation.

3.4. Inhibition of IGF-1R precludes drug washout-mediated cancer cell growth

Inhibition of AKT pathway leads to upregulation of receptor tyrosine kinases activities such as IGF-1R, insulin receptor, or HER3 [6]. Similarly, we found that washout of mTOR or PI3K inhibitors resulted in increased phosphorylation of IGF-1R β compared to control cells treated with DMSO (Fig. 4A). Inhibition of IGF-1R with NVP-AEW541 [25] prevented AKT overactivation following washout of PP242 or BKM-120 (Fig. 4B). In contrast, EGFR inhibitor erlotinib [26] or EGFR, Erb2, Erb3 inhibitor sapitinib [27] had no effect (Fig. 4B). Finally, increased cancer cell proliferation mediated by cessation of PP242 or BKM-120 was prevented by NVP-AEW541 (Fig. 4C). Taken together these results suggest that increased activity of IGF-1R following withdrawal of PP242 or BKM-120 upregulates cancer cell proliferation.

4. Discussion

Targeting the PI3K/AKT/mTOR signaling pathway in cancer has been less successful than expected. Limitations include activation of alternate proliferative signaling pathways due to abrogation of negative feedback loops following AKT or mTORC1 inhibition [6-10, 28]. In addition, transient inhibition of the pathway was documented. For instance, PI3K inhibitors block AKT activity with a progressive return to baseline after 48 hours due to PI3K independent activation mechanisms of AKT [29,

30]. Here, we further report that discontinuation of PI3K or mTOR inhibitors leads to AKT overactivation that promotes cancer cell proliferation (Fig. 2). Consistent with our observation, a proliferative rebound was noted following discontinuation of the PI3K inhibitor GDC-0941 and recovery of PI3K activity in mice bearing tumors [15]. Whereas an association between increased cancer cell proliferation and tumor growth was not tested in this study, another study showed increased cancer growth following cessation of the dual PI3K/mTOR inhibitor NVP-BEZ235 [31], suggesting that drug withdrawal promotes a more aggressive phenotype.

In HT-29 cells, upregulation of IGF-1R activity following drug withdrawal was responsible for signal transmission to AKT and stimulation of cancer cell proliferation (Fig. 4). Upregulation of the activity of growth factor receptors, not restricted to IGF-1R, has been demonstrated following inhibition of the PI3K/AKT/mTOR signaling pathway. For instance, inhibition of AKT leads to FOXO dependent transcription of growth factor receptors including HER3, IGF-1R, and IR [6]. Similarly, suppression of PI3K leads to increased HER2/HER3 signaling that counteracts the anti-cancer efficacy of PI3K inhibitors [7, 8]. Consequently, combining growth factor receptors inhibitors with PI3K or mTOR inhibitors or combining PI3K or mTOR inhibitors with AKT inhibitors provide stronger anti-cancer efficacy than monotherapies. Our data further suggest that upregulation of growth factor receptors signaling persists after discontinuation of mTOR and PI3K inhibitors and recovery of AKT activity, resulting in increased cancer cell proliferation. Accordingly, inhibition of growth factor receptors and of AKT should be performed following discontinuation of PI3K or mTOR inhibitors to prevent rebound cancer cell proliferation.

Several targeted therapies including PI3K and mTOR inhibitors are associated with significant toxicities upon continuous administration to patients [32]. Hence, new scheduling alternatives such as intermittent treatments need to be explored to limit these toxicities. In fact, transient pathway inhibition for example in the case of lapatinib has demonstrated similar anti-tumor response as continuous inhibition [13]. Similarly,

intermittent BCR-ABL inhibition with dasatinib achieves clinical remission in chronic myeloid leukemia patients [12]. Intermittent treatment with PI3K and mTOR inhibitors should however be carefully monitored as our results suggest that AKT overactivation following drug withdrawal promotes cancer cell growth.

Conflicts of interest

The authors declare no conflicting interests.

Funding

This work was supported by the Swiss Cancer League (KFS-3128-02-2013).

Figure Legends

Fig. 1. mTOR inhibitors or PI3K inhibitors washout increases AKT phosphorylation (**A**) SW480 and HT-29 colon cancer cells were treated for 24 hours with 5 μ M of PP242 (PP) or 5 μ M Ku-0063794 (Ku). Cell lysates were collected immediately or after one hour of drug washout and analyzed by immunoblotting for AKT and phospho-AKT (Ser 473). Three independent experiments were quantified by densitometry and relative phospho-AKT levels are depicted. * $p < 0.05$. (**B**) Huh-7 and 786-O were processed as under panel (A) and cell lysates were analyzed by immunoblotting for AKT and phospho-AKT (Ser 473). (**C**) HT-29 colon cancer cells were treated for 24 hours with PP242 (5 μ M) or DMSO as a control. Drug was then washed out and cell lysates were collected 0, 1, 6, 12, 24, 48, and 72 hours after washout and analyzed by immunoblotting for AKT and phospho-AKT (Ser 473). (**D**) HT-29 cancer cells were treated with 5 μ M of PP242 for 1, 6, or 24 hours and subjected or not to one hour drug washout. Cell lysates were collected and analyzed for AKT and phospho-AKT (Ser 473). (**E**) LS-174T colon cancer cells were treated for 24 hours with 500nM of BKM-120 (BKM), 500nM of PX-866 (PX) or 10 μ M of BYL719 (BYL). Cell lysates were collected immediately or after one hour of drug washout and analyzed by immunoblotting for phospho-AKT (Ser 473) and actin.

Fig. 2. Washout of mTOR or PI3K inhibitors promotes cancer cell growth. **(A)** Experimental design. SW480, HT-29, 786-O, or Huh-7 cancer cells were plated and treated 24 hours later with PP242 (5 μ M) or BKM-120 (500nM) for six hours. Drug washout was subsequently performed, and MTS proliferation assay or cell counting were performed after a further 48 hours. **(B)** MTS proliferation assay 48 hours post drug washout. Columns: Mean cell proliferation of three independent experiments expressed as percentage of DMSO treated cancer cells. Bars: SD. White columns: DMSO treated cells, grey columns: cell treated with PP242, and black columns: cells treated with BKM-120. * $p < 0.05$ compared to control cells. **(C)** Cell counting 48 hours post drug washout. Columns: Mean cell count expressed as percentage of DMSO treated cancer cells of three independent experiments. Bars: SD. * $p < 0.05$ compared to control cells.

Fig. 3. Targeting AKT blocks drug washout mediated cancer cell growth. SW480, HT-29, 786-O or Huh-7 were treated with DMSO, PP242 (5 μ M) or BKM-120 (500nM) for six hours. Drugs were washed out and cells were incubated with DMSO or MK-2206 (1 μ M) for 48 hours before performing an MTS proliferation assay. Columns: Mean cell proliferation of three independent experiments expressed as percentage of DMSO treated cancer cells. Bars: SD. White columns: DMSO treated cells, grey columns: cell treated with PP242, and black columns: cells treated with BKM-120. * $p < 0.05$ compared to control cells.

Fig. 4. Blocking IGF-1R prevents drug washout mediated cancer cell growth. **(A)** Washout of PI3K or mTOR inhibitors increases IGF-1R β phosphorylation. HT-29 cells were treated for six hours with DMSO, BYL719 (10 μ M) (BYL), BKM-120 (500nM) (BKM), PP242 (5 μ M) or Ku0063794 (5 μ M) (Ku). Following washout, cells were incubated for six hours, and cell lysates were collected and analyzed for IGF-1R β phosphorylation and actin. **(B)** IGF-1R inhibition with NVP-AEW541 blocks AKT phosphorylation mediated by washout of PP242 or BKM-120. HT-29 cells were treated with DMSO, PP242 (5 μ M) or BKM-120 (500mM) for 6 hours. Following drug washout,

cells were treated as indicated with sapitinib (2 μ M), NVP-AEW541 (1 μ M) (NVP-AEW) or erlotinib (1 μ M) for six hours, and AKT phosphorylation was assessed by Western blot in cell lysates. (C) NVP-AEW541 inhibits cancer cell proliferation induced by washout of PP242 or BKM-120. HT-29 cells were treated with DMSO, PP242 (5 μ M) or BKM-120 (500nM) for six hours. Following drug washout, cells were incubated with DMSO or NVP-AEW541 (1 μ M) for an additional 48 hours. Cell proliferation was assessed with an MTS proliferation assay. Columns: Mean cell proliferation of three independent experiments expressed as percentage of DMSO treated cancer cells. Bars: SD. White columns: DMSO treated cells, grey columns: cells treated with PP242, and black columns: cells treated with BKM-120. * $p < 0.05$ compared to control cells.

References

- [1] T.A. Baudino, Targeted Cancer Therapy: The Next Generation of Cancer Treatment, *Curr Drug Discov Technol*, 12 (2015) 3-20.
- [2] P.M. LoRusso, Inhibition of the PI3K/AKT/mTOR Pathway in Solid Tumors, *J Clin Oncol*, 34 (2016) 3803-3815.
- [3] C. Porta, C. Paglino, A. Mosca, Targeting PI3K/Akt/mTOR Signaling in Cancer, *Front Oncol*, 4 (2014) 64.
- [4] Y. Zhang, P. Kwok-Shing Ng, M. Kucherlapati, F. Chen, Y. Liu, Y.H. Tsang, G. de Velasco, K.J. Jeong, R. Akbani, A. Hadjipanayis, A. Pantazi, C.A. Bristow, E. Lee, H.S. Mahadeshwar, J. Tang, J. Zhang, L. Yang, S. Seth, S. Lee, X. Ren, X. Song, H. Sun, J. Seidman, L.J. Luquette, R. Xi, L. Chin, A. Protopopov, T.F. Westbrook, C.S. Shelley, T.K. Choueiri, M. Ittmann, C. Van Waes, J.N. Weinstein, H. Liang, E.P. Henske, A.K. Godwin, P.J. Park, R. Kucherlapati, K.L. Scott, G.B. Mills, D.J. Kwiatkowski, C.J. Creighton, A Pan-Cancer Proteogenomic Atlas of PI3K/AKT/mTOR Pathway Alterations, *Cancer Cell*, 31 (2017) 820-832 e823.

- [5] J. Rodon, J. Taberero, Improving the Armamentarium of PI3K Inhibitors with Isoform-Selective Agents: A New Light in the Darkness, *Cancer Discov*, 7 (2017) 666-669.
- [6] S. Chandarlapaty, A. Sawai, M. Scaltriti, V. Rodrik-Outmezguine, O. Grbovic-Huezo, V. Serra, P.K. Majumder, J. Baselga, N. Rosen, AKT inhibition relieves feedback suppression of receptor tyrosine kinase expression and activity, *Cancer Cell*, 19 (2011) 58-71.
- [7] V. Serra, M. Scaltriti, L. Prudkin, P.J. Eichhorn, Y.H. Ibrahim, S. Chandarlapaty, B. Markman, O. Rodriguez, M. Guzman, S. Rodriguez, M. Gili, M. Russillo, J.L. Parra, S. Singh, J. Arribas, N. Rosen, J. Baselga, PI3K inhibition results in enhanced HER signaling and acquired ERK dependency in HER2-overexpressing breast cancer, *Oncogene*, 30 (2011) 2547-2557.
- [8] A. Chakrabarty, V. Sanchez, M.G. Kuba, C. Rinehart, C.L. Arteaga, Feedback upregulation of HER3 (ErbB3) expression and activity attenuates antitumor effect of PI3K inhibitors, *Proc Natl Acad Sci U S A*, 109 (2012) 2718-2723.
- [9] A. Carracedo, L. Ma, J. Teruya-Feldstein, F. Rojo, L. Salmena, A. Alimonti, A. Egia, A.T. Sasaki, G. Thomas, S.C. Kozma, A. Papa, C. Nardella, L.C. Cantley, J. Baselga, P.P. Pandolfi, Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer, *J Clin Invest*, 118 (2008) 3065-3074.
- [10] K.E. O'Reilly, F. Rojo, Q.B. She, D. Solit, G.B. Mills, D. Smith, H. Lane, F. Hofmann, D.J. Hicklin, D.L. Ludwig, J. Baselga, N. Rosen, mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt, *Cancer Res*, 66 (2006) 1500-1508.
- [11] E. Pons-Tostivint, B. Thibault, J. Guillermet-Guibert, Targeting PI3K Signaling in Combination Cancer Therapy, *Trends Cancer*, 3 (2017) 454-469.
- [12] N.P. Shah, C. Kasap, C. Weier, M. Balbas, J.M. Nicoll, E. Bleickardt, C. Nicaise, C.L. Sawyers, Transient potent BCR-ABL inhibition is sufficient to commit chronic myeloid leukemia cells irreversibly to apoptosis, *Cancer Cell*, 14 (2008) 485-493.

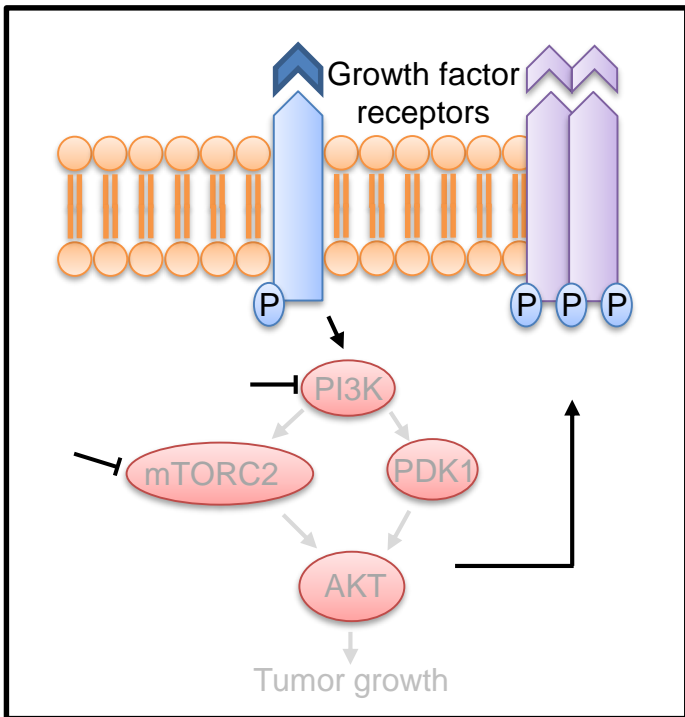
- [13] H.K. Chew, G. Somlo, P.C. Mack, B. Gitlitz, R. Gandour-Edwards, S. Christensen, H. Linden, L.J. Solis, X. Yang, A.M. Davies, Phase I study of continuous and intermittent schedules of lapatinib in combination with vinorelbine in solid tumors, *Ann Oncol*, 23 (2012) 1023-1029.
- [14] O. Abdel-Wahab, V.M. Klimek, A.A. Gaskell, A. Viale, D. Cheng, E. Kim, R. Rampal, M. Bluth, J.J. Harding, M.K. Callahan, T. Merghoub, M.F. Berger, D.B. Solit, N. Rosen, R.L. Levine, P.B. Chapman, Efficacy of intermittent combined RAF and MEK inhibition in a patient with concurrent BRAF- and NRAS-mutant malignancies, *Cancer Discov*, 4 (2014) 538-545.
- [15] W. Yang, S.R. Hosford, L.M. Dillon, K. Shee, S.C. Liu, J.R. Bean, L. Salphati, J. Pang, X. Zhang, M.A. Nannini, E. Demidenko, D. Bates, L.D. Lewis, J.D. Marotti, A.R. Eastman, T.W. Miller, Strategically Timing Inhibition of Phosphatidylinositol 3-Kinase to Maximize Therapeutic Index in Estrogen Receptor Alpha-Positive, PIK3CA-Mutant Breast Cancer, *Clin Cancer Res*, 22 (2016) 2250-2260.
- [16] D.M. Geynisman, K.E. Wickersham, Adherence to targeted oral anticancer medications, *Discov Med*, 15 (2013) 231-241.
- [17] A.H. Partridge, J. Avorn, P.S. Wang, E.P. Winer, Adherence to therapy with oral antineoplastic agents, *J Natl Cancer Inst*, 94 (2002) 652-661.
- [18] B. Apsel, J.A. Blair, B. Gonzalez, T.M. Nazif, M.E. Feldman, B. Aizenstein, R. Hoffman, R.L. Williams, K.M. Shokat, Z.A. Knight, Targeted polypharmacology: discovery of dual inhibitors of tyrosine and phosphoinositide kinases, *Nat Chem Biol*, 4 (2008) 691-699.
- [19] J.M. Garcia-Martinez, J. Moran, R.G. Clarke, A. Gray, S.C. Cosulich, C.M. Chresta, D.R. Alessi, Ku-0063794 is a specific inhibitor of the mammalian target of rapamycin (mTOR), *Biochem J*, 421 (2009) 29-42.
- [20] D.D. Sarbassov, D.A. Guertin, S.M. Ali, D.M. Sabatini, Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex, *Science*, 307 (2005) 1098-1101.

- [21] N.T. Ihle, R. Williams, S. Chow, W. Chew, M.I. Berggren, G. Paine-Murrieta, D.J. Minion, R.J. Halter, P. Wipf, R. Abraham, L. Kirkpatrick, G. Powis, Molecular pharmacology and antitumor activity of PX-866, a novel inhibitor of phosphoinositide-3-kinase signaling, *Mol Cancer Ther*, 3 (2004) 763-772.
- [22] D. Koul, J. Fu, R. Shen, T.A. LaFortune, S. Wang, N. Tiao, Y.W. Kim, J.L. Liu, D. Ramnarian, Y. Yuan, C. Garcia-Echeverria, S.M. Maira, W.K. Yung, Antitumor activity of NVP-BKM120--a selective pan class I PI3 kinase inhibitor showed differential forms of cell death based on p53 status of glioma cells, *Clin Cancer Res*, 18 (2012) 184-195.
- [23] P. Furet, V. Guagnano, R.A. Fairhurst, P. Imbach-Weese, I. Bruce, M. Knapp, C. Fritsch, F. Blasco, J. Blanz, R. Aichholz, J. Hamon, D. Fabbro, G. Caravatti, Discovery of NVP-BYL719 a potent and selective phosphatidylinositol-3 kinase alpha inhibitor selected for clinical evaluation, *Bioorg Med Chem Lett*, 23 (2013) 3741-3748.
- [24] H. Hirai, H. Sootome, Y. Nakatsuru, K. Miyama, S. Taguchi, K. Tsujioka, Y. Ueno, H. Hatch, P.K. Majumder, B.S. Pan, H. Kotani, MK-2206, an allosteric Akt inhibitor, enhances antitumor efficacy by standard chemotherapeutic agents or molecular targeted drugs in vitro and in vivo, *Mol Cancer Ther*, 9 (2010) 1956-1967.
- [25] C. Garcia-Echeverria, M.A. Pearson, A. Marti, T. Meyer, J. Mestan, J. Zimmermann, J. Gao, J. Brueggen, H.G. Capraro, R. Cozens, D.B. Evans, D. Fabbro, P. Furet, D.G. Porta, J. Liebetanz, G. Martiny-Baron, S. Ruetz, F. Hofmann, In vivo antitumor activity of NVP-AEW541-A novel, potent, and selective inhibitor of the IGF-IR kinase, *Cancer Cell*, 5 (2004) 231-239.
- [26] V.A. Pollack, D.M. Savage, D.A. Baker, K.E. Tsaparikos, D.E. Sloan, J.D. Moyer, E.G. Barbacci, L.R. Pustilnik, T.A. Smolarek, J.A. Davis, M.P. Vaidya, L.D. Arnold, J.L. Doty, K.K. Iwata, M.J. Morin, Inhibition of epidermal growth factor receptor-associated tyrosine phosphorylation in human carcinomas with CP-358,774: dynamics of receptor inhibition in situ and antitumor effects in athymic mice, *J Pharmacol Exp Ther*, 291 (1999) 739-748.

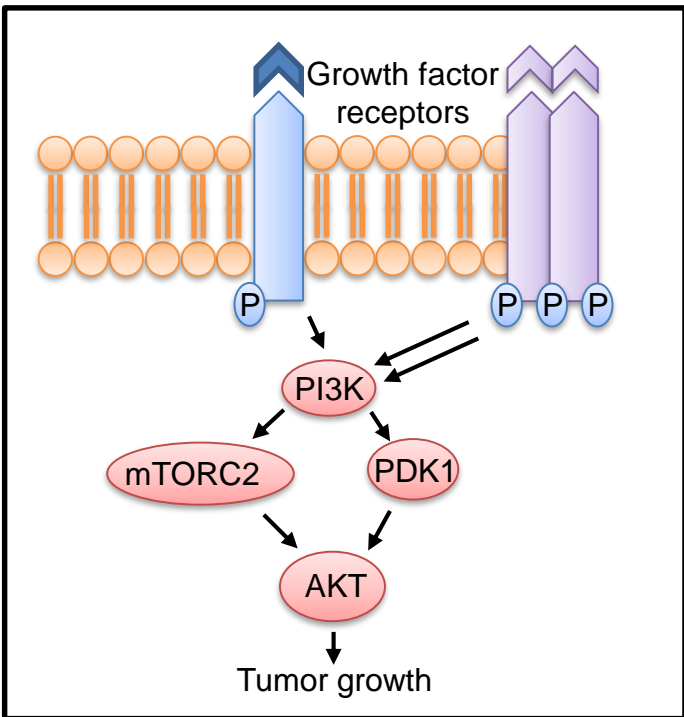
- [27] D.M. Hickinson, T. Klinowska, G. Speake, J. Vincent, C. Trigwell, J. Anderton, S. Beck, G. Marshall, S. Davenport, R. Callis, E. Mills, K. Grosios, P. Smith, B. Barlaam, R.W. Wilkinson, D. Ogilvie, AZD8931, an equipotent, reversible inhibitor of signaling by epidermal growth factor receptor, ERBB2 (HER2), and ERBB3: a unique agent for simultaneous ERBB receptor blockade in cancer, *Clin Cancer Res*, 16 (2010) 1159-1169.
- [28] A. Bosch, Z. Li, A. Bergamaschi, H. Ellis, E. Toska, A. Prat, J.J. Tao, D.E. Spratt, N.T. Viola-Villegas, P. Castel, G. Minuesa, N. Morse, J. Rodon, Y. Ibrahim, J. Cortes, J. Perez-Garcia, P. Galvan, J. Grueso, M. Guzman, J.A. Katzenellenbogen, M. Kharas, J.S. Lewis, M. Dickler, V. Serra, N. Rosen, S. Chandarlapaty, M. Scaltriti, J. Baselga, PI3K inhibition results in enhanced estrogen receptor function and dependence in hormone receptor-positive breast cancer, *Sci Transl Med*, 7 (2015) 283ra251.
- [29] M. Dufour, A. Dormond-Meuwly, C. Pythoud, N. Demartines, O. Dormond, Reactivation of AKT signaling following treatment of cancer cells with PI3K inhibitors attenuates their antitumor effects, *Biochem Biophys Res Commun*, 438 (2013) 32-37.
- [30] S. Faes, O. Dormond, PI3K and AKT: Unfaithful Partners in Cancer, *Int J Mol Sci*, 16 (2015) 21138-21152.
- [31] D. Roulin, L. Waselle, A. Dormond-Meuwly, M. Dufour, N. Demartines, O. Dormond, Targeting renal cell carcinoma with NVP-BEZ235, a dual PI3K/mTOR inhibitor, in combination with sorafenib, *Mol Cancer*, 10 (2011) 90.
- [32] E. Toska, J. Baselga, Pharmacology in the Era of Targeted Therapies: The Case of PI3K Inhibitors, *Clin Cancer Res*, 22 (2016) 2099-2101.

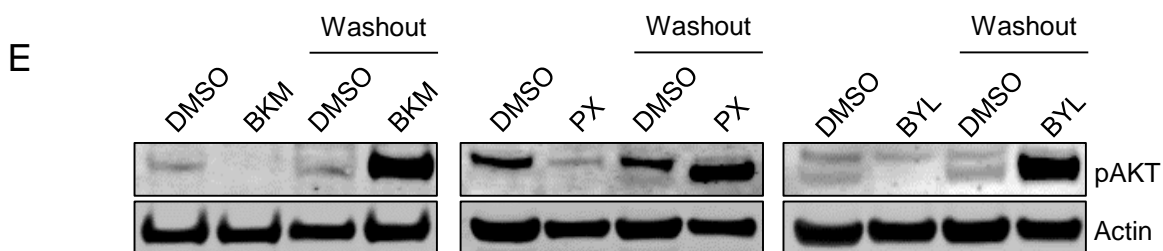
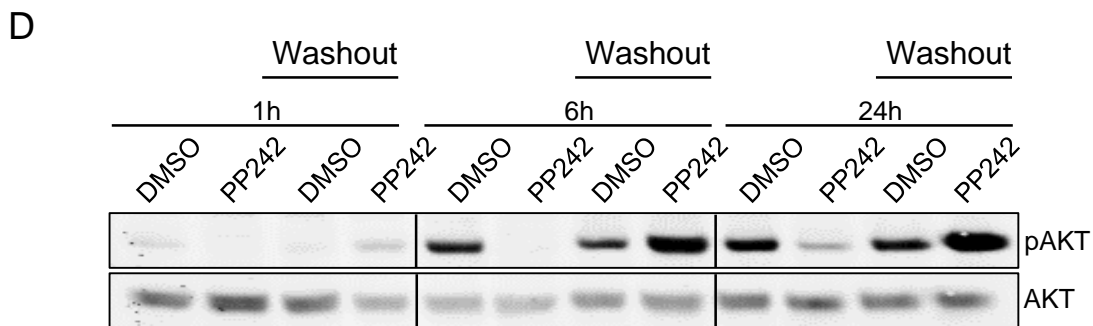
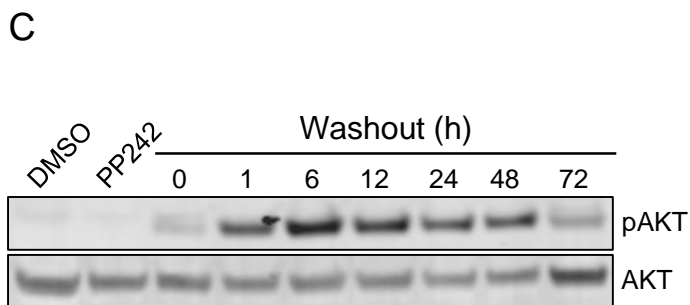
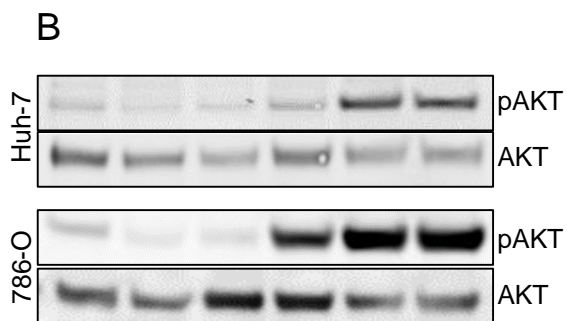
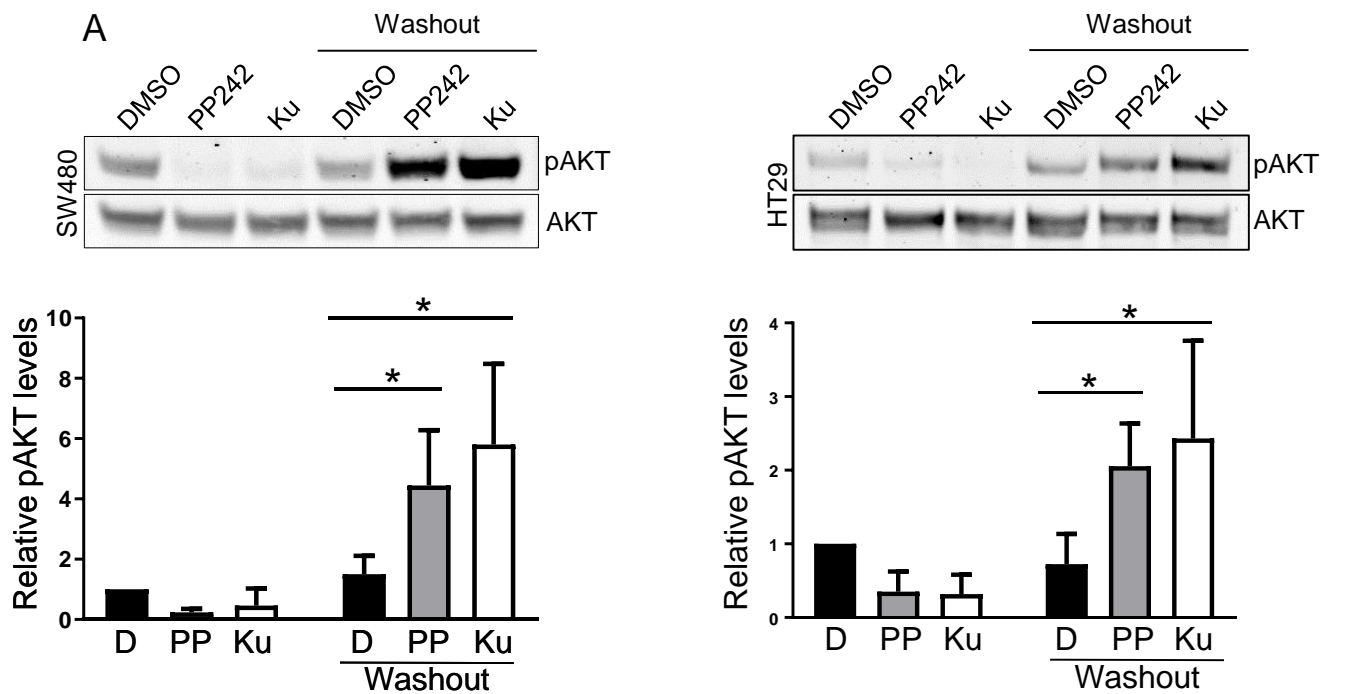
- Discontinuation of PI3K or mTOR inhibitors induces AKT overactivation.
- Increased AKT activity following drug washout promotes cancer cell growth.
- Targeting AKT following cessation of mTOR or PI3K inhibitors provides anti-cancer benefits.

Treatment with PI3K or mTOR inhibitors

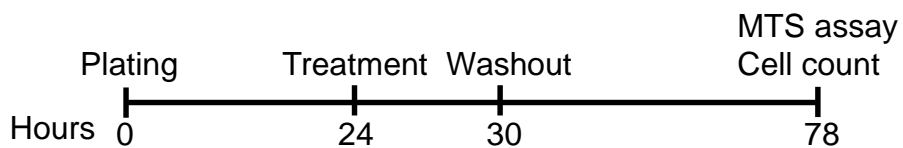


Drug withdrawal

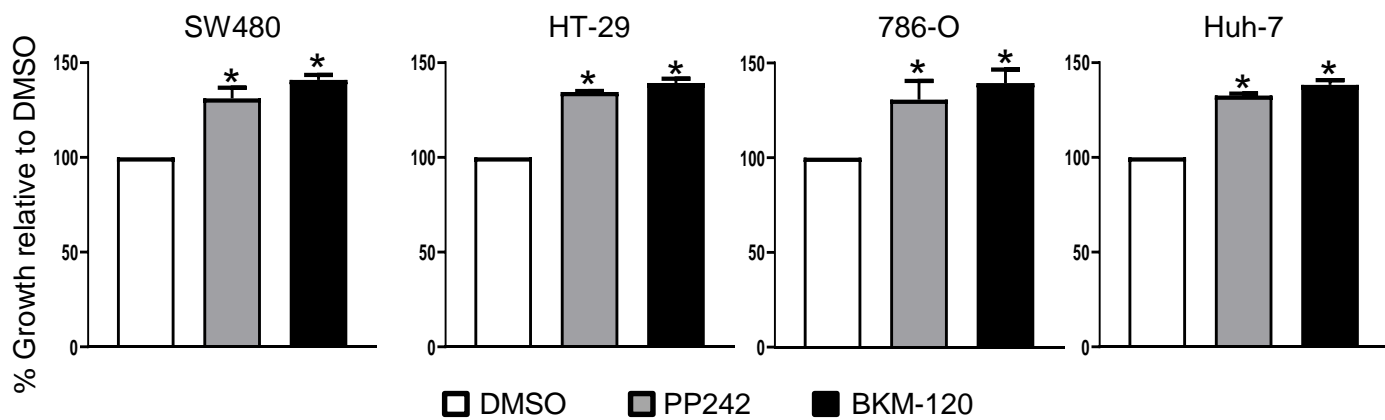




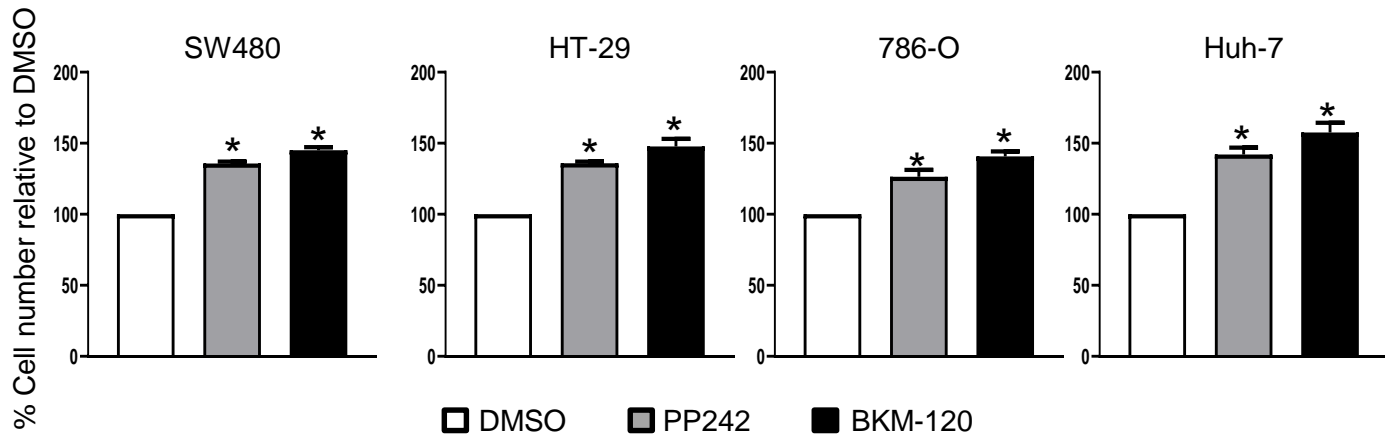
A



B



C



A

