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**Targeting the genetic alterations of the PI3K-AKT-mTOR pathway: its potential use in the treatment of bladder cancers.**

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**ABSTRACT** (250 words)

Urothelial carcinoma of the bladder is the most frequent tumor of the urinary tract and represents the fifth cause of death by cancer worldwide. The current first line chemotherapy is a combination of cisplatin and gemcitabine with median survival not exceeding 15 months. Vinflunine is the only drug approved by EMEA as second-line treatment and few progresses have been made for the past 20 years to increase the survival of metastatic patients, especially those who are not eligible for cisplatin-based regimen. The recent studies characterizing the genetic background of urothelial cancers of the bladder, revealed chromosomal alterations that are not seen at the same level in other types of cancers. This is especially the case for mutations of genes involved in the PI3K/AKT/mTOR signaling pathway that occupies a major place in the etiology of these tumors. Here, we describe the mutations leading to constitutive activation of the PI3K/AKT/mTOR pathway and discuss the potential use of the different classes of PI3K/AKT/mTOR inhibitors in the treatment of urothelial bladder cancers. Despite the recent pivotal study evidencing specific mutations of TSC1 in bladder cancer patients responding to everolimus and the encouraging results obtained with other derivatives than rapalogs, few clinical trials are ongoing in bladder cancers. Because of the genetic complexity of these tumors, the cross-talks of the PI3K/AKT/mTOR pathway with other pathways, and the small number of eligible patients, it will be of utmost importance to carefully choose the drugs or drug combinations to be further tested in the clinic.

**Keywords** :Urothelial bladder cancer, PI3K, AKT, PTEN, mTOR, TSC1

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**Abbreviations:** AMPK: AMP-activated protein kinase; Bad: B cell leukemia/lymphoma-2 associated death agonist; Bcl-2: B cell leukemia-2; Bim: B cell leukemia/lymphoma-2 interacting mediator of cell death; CREB: cyclic AMP response element-binding protein; Deptor: DEP domain containing mTOR-interacting protein; eIF-4E: eukaryotic translation initiation factor 4E; FKBP38: FK506 Binding Protein 38; Foxo3a: Forkhead box O3; GSK3 $\beta$ : glycogen synthase kinase-3 $\beta$ ; HIF-1 $\alpha$ : Hypoxia-inducible transcription factor 1 $\alpha$ ; Hsp70: heat shock protein 70; IKK: inhibitor of  $\kappa$ B kinases; IRS1: Insulin Receptor Substrate 1; LKB1: Liver kinase B1; Mcl-1: myeloid cell leukemia-1; MDM2: murine double minute 2; MDR1: multidrug resistance protein 1; mLST8: mammalian Lethal-with-Sec-Thirteen protein 8; mTORC: mammalian target of rapamycin complex; NF- $\kappa$ B: nuclear factor  $\kappa$ B; p27<sup>Kip1</sup>: p27 cyclin-dependent kinase inhibitor; PDGF: Platelet-derived growth factor; PDK1: phosphoinositide-dependent kinase 1; PHLPP: PH domain leucine-rich repeat protein phosphatase; PI3K: phosphatidylinositol 3-kinase; PIP2: phosphatidylinositol-4,5-bisphosphate; PIP3: phosphatidylinositol-3,4,5-triphosphate; PRAS40: proline-rich Akt substrate of 40 kDa; Protor: Protein observed with Rictor; PTEN: phosphatase and tensin homolog; Puma: p53 upregulated mediator of apoptosis; Raptor: Regulatory associated protein of mTOR; RHEB: Ras homolog enriched in brain; Rictor: Rapamycin-insensitive companion of mTOR; RTK: receptor tyrosine kinase; SGK: serum- and glucocorticoid-induced protein kinase; SIN-1: stress-activated protein kinase-interacting protein 1; TSC: tuberous sclerosis complex; ULK1: unc-51-like kinase 1; VEGF: Vascular endothelial growth factor; XIAP: X-linked inhibitor of apoptosis

## 1. Introduction

The phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR pathway (Fig.1) is an essential pathway for various cellular processes including cell growth, cell survival, cell motility, angiogenesis, as well as cell metabolism (Bartholomeusz & Gonzalez-Angulo, 2012; Courtney, Corcoran, & Engelman, 2010; Knowles, Platt, Ross, & Hurst, 2009; McCubrey, Steelman, Chappell, Abrams, Franklin, et al., 2012; McCubrey, Steelman, Chappell, Abrams, Montalto, et al., 2012; Populo, Lopes, & Soares, 2012; Shaw & Cantley, 2006). It is also involved in the resistance to “conventional” chemotherapeutic agents by modulating the expression of efflux pumps from the ABC transporters family (H. A. Burris, 3rd, 2013; Tazzari, et al., 2007). Its constitutive activation, that is mainly due to genetic alterations of key genes involved in this pathway, is observed in various types of cancers (see (P. Liu, Cheng, Roberts, & Zhao, 2009) for review), including urothelial bladder cancers (Bambury & Rosenberg, 2013; Knowles, et al., 2009). As such it represents an attractive target for cancer therapy (Chang, et al., 2003; P. Liu, et al., 2009). Urothelial carcinoma of the bladder represents the most frequent tumor of the urinary tract and leads to a fatal issue for approximately 150,000 patients each year worldwide (Ploeg, Aben, & Kiemeny, 2009). In the case of advanced metastatic forms of the disease, chemotherapies such as a combination of cisplatin with gemcitabine or combination of cisplatin with methotrexate, vinblastine and adriamycin (MVAC protocol) are used, with a 5-year survival rate not exceeding 15% (Sio, Ko, Gudena, Verma, & Chaudhary, 2014; von der Maase, et al., 2005), which stresses the urgent need for alternative therapeutics. The growing number of studies reporting new alterations of this pathway leading to its constitutive activation, have led to the identification of new generations of tyrosine kinase inhibitors specifically targeting such mutations in various tumor types with significant improvement in terms of tumor responses (Bambury & Rosenberg, 2013; Bartholomeusz & Gonzalez-Angulo, 2012; Bitting & Armstrong, 2013; H. A. Burris, 3rd, 2013; McCubrey, Steelman, Chappell, Abrams, Montalto, et al., 2012). In this review, we will provide an update of the genomic alterations that have been specifically identified in bladder cancers. We will also discuss the use of inhibitors targeting the PI3K/AKT/mTOR pathway in patients with urothelial cancers, taking into account the known limitations resulting from feed-back loops and the delicate issue of cross-talks with other essential pathways regulating cell proliferation such as the RAS/RAF/MEK/ERK pathway (Courtney, et al., 2010; Markman, Dienstmann, & Taberero, 2010; McCubrey, Steelman, Chappell, Abrams, Franklin, et al.,

2012)(Fig.1). This latter aspect is certainly representing one of the major challenges of modern pharmacology regarding the rational choice for synergistic drug combinations.

## **2. The PI3K/AKT/mTOR pathway and its cross-talks with other pathways**

The PI3K/AKT/mTOR pathway is initiated by the tyrosine phosphorylation of the intracellular domain of receptor tyrosine kinases (RTKs) resulting from interaction of RTKs with their respective ligands at the cell surface (Fig.1). This induces the activation of class IA phosphatidylinositol 3-kinase (PI3K) as second messenger. Class IA PI3K is a heterodimeric kinase composed of two subunits: the catalytic subunit p110 and the regulatory subunit p85. There are four isoforms of p110 ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ) encoded by PIK3CA, PIK3CB, PIK3CG, and PIK3CD genes, respectively, the delta isoforms being primarily found in lymphocytes (Engelman, Luo, & Cantley, 2006; Katso, et al., 2001). Similarly, the three isoforms p85 $\alpha$ ,  $\beta$  and  $\gamma$  are encoded by three distinct genes, PIK3R1, PIK3R2, and PIK3R3 (Engelman, et al., 2006; Katso, et al., 2001). p85 contains a Src homology domain (SH2) that allows recognition and binding to activated RTK phosphotyrosine. This binding can be direct but can also be mediated by adaptor proteins such as IRS-1 (Insulin Receptor Substrate 1) (Carpenter, et al., 1993). It results in the activation of the p110 catalytic subunit and its relocalization to the inner layer of the cytoplasmic phospholipid membrane where it phosphorylates the 3'-hydroxyl group of phosphatidylinositol-4,5-bisphosphate (PIP2) to generate phosphatidylinositol-3,4,5-triphosphate (PIP3) (Fig.1) (Cantley, 2002). PIP3 plays a pivotal role in further signaling of the pathway as it is a substrate of various protein kinases containing a pleckstrin homology (PH) domain such as the serine-threonine kinase AKT and the phosphoinositide-dependent kinase 1 (PDK1) (Cantley, 2002). This interaction leads to the recruitment of AKT to the cell membrane where it is phosphorylated on both threonine 308 by PDK1 (Alessi, et al., 1997; Stokoe, et al., 1997) and serine 473 by the mTORC2 complex via a positive feedback loop (Sarbasov, Guertin, Ali, & Sabatini, 2005), leading to its full activation. Dephosphorylation of serine 473 can be achieved by the PH domain leucine-rich repeat protein phosphatase (PHLPP) resulting in cell growth inhibition (Gao, Furnari, & Newton, 2005). PIP3 can also bind the tumor suppressor phosphatase and tensin homolog (PTEN) that dephosphorylates PIP3 to

PIP2 (Fig.1). As such, PTEN represents a key negative regulator of the PI3K/AKT/mTOR signaling (Chalhoub & Baker, 2009).

Activated AKT can phosphorylate a myriad of downstream effectors regulating a variety of essential processes such as protein synthesis, cell metabolism, cell growth/proliferation, as well as cell survival and resistance to various exogenous stresses (Hay, 2005; Steelman, Stadelman, et al., 2008). Providing an exhaustive list of all these targets and describing the functional consequences of their phosphorylation in terms of activation or inhibition is beyond the scope of this review. Only AKT substrates that could be relevant for cell response to anticancer drugs will be cited. Among them, AKT modulates the activity of multiple transcription factors such as CREB (cyclic AMP response element-binding protein), the phosphorylation of which increases the expression of antiapoptotic genes such as Bcl-2 or Mcl-1 (Du & Montminy, 1998). Activated AKT also inhibits the transcriptional activity of Foxo3a (Forkhead box O3) leading to reduced expression of several pro-apoptotic genes such as caspase-9, caspase-3, Bim, Bad, Noxa, or Puma (You, et al., 2006) or the expression of p27 cyclin-dependent kinase inhibitor (p27<sup>Kip1</sup>) that is required for cell cycle arrest in G1 (Liang, et al., 2002). It can also result in increased expression of anti-apoptotic genes such as Bcl-2, XIAP, or NF- $\kappa$ B (Dan, et al., 2008). The transcriptional activity of NF- $\kappa$ B is also under the control of AKT that directly phosphorylates the inhibitor of  $\kappa$ B kinase (IKK). This leads to the phosphorylation of the inhibitory protein I $\kappa$ B, inducing its degradation and the release of NF- $\kappa$ B that translocates into the nucleus where it regulates the expression of its target genes (Ozes, et al., 1999). This mechanism has been implicated in the modulation of ABBC1 expression, a membrane transporter (formerly called MRP1) playing a major role in drug resistance to various anticancer agents (Tazzari, et al., 2007).

AKT also inhibits the function of the tuberous sclerosis TSC1/2 complex. Tuberous sclerosis is a rare dominant autosomal disorder resulting from mutations in one of the two genes TSC1 (hamartin) or TSC2 (tuberin), leading to the occurrence of non-malignant tumors in different organs including brain, retina, kidney, heart, and lung (Crino, Nathanson, & Henske, 2006). AKT phosphorylates TSC2 that leads to the disruption of its interaction with TSC1 (Inoki, Li, Zhu, Wu, & Guan, 2002) and further blockage of the GAP (GTPase activating protein) function of the complex which in turn prevents the conversion of the active form of RHEB (Ras homolog enriched in brain) bound to GTP in its inactive form bound to GDP. Then, active RHEB phosphorylates mTOR (mammalian target of rapamycin), the



third master player of this signaling pathway, at serine 2448 (Inoki, Li, Xu, & Guan, 2003). The TOR gene was originally discovered by a genetic screen in yeast *Saccharomyces cerevisiae* looking for resistant clones to the fungicide rapamycin (Heitman, Movva, & Hall, 1991), and its homologs were further characterized in many other species including mammals. mTOR is a high molecular weight serine/threonine kinase from the PIKK (PI3K-related protein kinase) family (Zoncu, Efeyan, & Sabatini, 2011). It is part of two complexes, mTORC1 and mTORC2 that differ from their composition and have distinct functional properties, starting with responsiveness to rapamycin treatment, mTORC1 being the most sensitive to the drug. In mTORC1, TOR associates with PRAS40 (proline-rich Akt substrate 40 kDa), mLST8 (mammalian Lethal-with-Sec-Thirteen 8), FKBP38 (FK506 Binding Protein 38), Raptor (Regulatory associated protein of mTOR), and Deptor (DEP domain containing mTOR-interacting protein) proteins whereas it interacts with mSIN1 (stress-activated protein kinase-interacting protein 1), mLST8, Rictor (Rapamycin-insensitive companion of mTOR), Protor, Deptor, and the heat shock protein Hsp70 in the mTORC2 complex (Fig.1)(Populo, et al., 2012; Zoncu, et al., 2011). Raptor serves as a scaffold protein to recruit mTORC1 substrates (Hara, et al., 2002; D. H. Kim, et al., 2002). Depending on the complex mLST8 regulates the interaction of mTOR with Raptor or Rictor (Zeng, et al., 2007) and activates its kinase activity(D. H. Kim, et al., 2003). Conversely, PRAS40 or Deptor have been described to exert a negative regulation on their respective complexes (Peterson, et al., 2009; Sancak, et al., 2007). PRAS40 can be directly phosphorylated by AKT that results in the inhibition of mTORC1 activation by Rheb (Sancak, et al., 2007). Similarly, FKBP38 was also proposed to play an inhibitory role on mTORC1 (Sato, Nakashima, Guo, & Tamanoi, 2009). Phosphorylation of AKT at serine 473 by mTORC2 depends on mSIN1 (Frias, et al., 2006) and is also regulated by the heat-shock protein Hsp70 (Martin, Masri, Bernath, Nishimura, & Gera, 2008).

As mentioned earlier, mTORC1 and mTORC2 complexes differently contribute to the global effect of PI3K/AKT/mTOR pathway activation on cell growth and the maintenance of cellular homeostasis. mTORC1 signaling is primarily mediated via the regulation of various factors involved in translation (Populo, et al., 2012; Zoncu, et al., 2011). Among them, mTORC1 phosphorylates the serine/threonine kinase S6K that leads to the phosphorylation of the 40S ribosomal protein S6 resulting in increased translation of S6K target genes (Hornberger, Sukhija, Wang, & Chien, 2007). mTORC1 also phosphorylates 4E-BP1 favoring its dissociation from the eukaryotic translation initiation factor 4E (eIF-4E), leading to the abrogation of its inhibitory effect on translation initiation (Pause, et

al., 1994). Regulation of translation by mTORC1 is highly sensitive to nutrient (amino-acids, glucose) levels and is tightly regulated by the two other kinases AMPK (AMP-activated protein kinase) and ULK1(unc-51-like kinase 1), the mammalian homolog of Atg1 that initiates the catabolic process of autophagy(J. Kim, Kundu, Viollet, & Guan, 2011; Nazio, et al., 2013). When enough nutrients are present, phosphorylation of ULK1 on S757 by mTORC1 prevents its interaction with AMPK and prevents autophagy (J. Kim, et al., 2011; Nazio, et al., 2013). In deprivation conditions,AMPK interacts with and phosphorylates ULK1 at other sites leading to the initiation of autophagy that is necessary for the restoration of nutrient levels(Egan, Kim, Shaw, & Guan, 2011). In parallel, AMPK phosphorylates TSC2 that inhibits mTORC1 activity, a mechanism that is further amplified by the phosphorylation of AMPK by the liver kinase B1 (LKB1) (Inoki, Zhu, & Guan, 2003).mTORC1 also plays an indirect role in angiogenesis by regulating the translation of HIF-1 $\alpha$  (Hypoxia-inducible transcription factor 1 $\alpha$ ) and the expression of its target genes encoding for essential angiogenic factors such as VEGF and PDGF (Manning & Cantley, 2003). Importantly, mTORC1 is capable to activate PI3K by a negative feed-back loop implicating the activation of S6K that in turns inhibits IRS-1 phosphorylation at Ser302 (Harrington, et al., 2004) (Fig. 1).

Probably because of its “resistance” to rapamycin, fewer studies have investigated the functional roles of the mTORC2 complex, but a growing number of studies provide evidences for its role in the regulation of multiple processes and its cross-talk with mTORC1 complex. mTORC2 was first identified as a key regulator of cytoskeleton organization and cell shape (Jacinto, et al., 2004), strengthening its potential role in cell migration, motility and tumor metastases (Zhou & Huang, 2011). PKC $\alpha$ , a kinase from the AGC (protein kinase A/protein kinase G/Protein kinase C) family is involved in this mechanism (Sarbassov, et al., 2004), but it is probable that other mTORC2 effectors could also contribute to this regulation. Other AGC kinases are also substrates of TORC2, including AKT (see above) and the serum and glucocorticoid-induced kinase 1 (SGK1) (Garcia-Martinez & Alessi, 2008), the phosphorylation of which leads to the regulation of sodium transport via the epithelial Na<sup>+</sup> channel (ENaC) (Bogusz, Brickley, Pew, & Conzen, 2006). Similarly to mTORC1, mTORC2 also regulates translationthrough its direct association with ribosomal proteins and the stabilization of newly synthesized polypeptides, including Akt(Oh, et al., 2010). This process is inhibited in the presence of dual inhibitors of mTORC1 and mTORC2 (Carayol, et al., 2010; Oh, et al., 2010; Yu, et al., 2009).

mTORC2 is also involved in metabolism and in autophagy, although these roles have not been as clearly established than for mTORC1 (Oh & Jacinto, 2011).

As shown in figure 1, RTKs activate both PI3K/AKT/mTOR and MAPK/ERK pathways that positively and negatively regulate each other. The molecular mechanisms involved in these cross-talks have been reviewed elsewhere and clearly demonstrate their importance in cell survival to inhibitors targeting either of these pathways (Carracedo, Baselga, & Pandolfi, 2008; Mendoza, Er, & Blenis, 2011). It was demonstrated that ERK could phosphorylate TSC2 and suppresses its tumor-suppressive function (Ma, Chen, Erdjument-Bromage, Tempst, & Pandolfi, 2005). Inhibition of mTORC1 was also shown to activate the MAPK/ERK pathway via a S6K-PI3K-Ras negative feedback loop (Carracedo, Ma, et al., 2008). This served as a rationale to test whether the effects of mTOR inhibitors could be potentiated by the addition of MEK1/2 inhibitors. Such a combination induced synergistic effects as compared to each inhibitor used separately in several in vitro and xenograft models (Carracedo, Baselga, et al., 2008).

By controlling such a wide range of ubiquitous cellular processes that are essential for the development and dissemination of cancer cells, it is not surprising that the PI3K/AKT/mTOR signaling pathway remains one of the most attractive target for the development of new anticancer agents. This is of particular importance in the context of alterations of specific genes involved in this pathway. As it will be described further, these “actionable” mutations have been identified at much higher rates in bladder cancers as compared to other cancer types, further reinforcing the interest of using inhibitors of this pathway to kill tumor cells in a more selective way.

### **3. Bladder cancers**

#### **3.1 Epidemiology of bladder cancer**

Bladder cancer is the ninth most common cancer worldwide with approximately 380,000 new cases and 150,000 deaths in 2008 (Jemal, et al., 2011). It is the second cancer of the genito-urinary tract, just behind prostate cancer and it occurs at a median age of 60 years old. The highest incidence rates are found in industrialized areas such as Europe and Northern America, but also occur in North African countries. Various risk factors with different impact on incidence have been associated with urothelial

cancers. It is now admitted that smoking is the most important one, as tobacco smoke contains various carcinogens including  $\beta$ -naphthylamine and PAH (Polycyclic Aromatic Hydrocarbons). aromatic amines are essentially metabolized by the liver and metabolites are excreted via the urinary tract, leading to their retention into the bladder where they could exert their carcinogenic effects. It is known that bladder cancer incidence is much higher in men (approximately 4-fold higher than in women) (Fajkovic, et al., 2011), a difference that was also evidenced in animal models (rats and mice) treated with nitrosamine derivatives (Bertram & Craig, 1972; Okajima, Hiramatsu, Iriya, Ijuin, & Matsushima, 1975). This gender disparity was first attributed to the higher proportion of smokers among men during the past century, but it turned out that tobacco could not completely explain such a disparity. Indeed, it was shown that age-standardized incidence of bladder cancer risk in never-smokers was also higher in men (Stenzl, et al., 2011), indicating that other risk factors may also be involved. These include exposure to other carcinogens or aromatic amines (pesticides in particular) through environmental and occupational exposures (Burger, et al., 2013). Implication of hormonal factors has also been demonstrated in animal models in which sex difference of bladder cancer development could be abolished by castrating males or by treating females with testosterone (Bertram & Craig, 1972). A link has also been established with several genetic factors such as the status of enzymes involved in the metabolism of carcinogens. This is especially the case for the NAT enzymes (N-acetyltransferase 1 and 2) for which slow acetylating activity is associated with increased bladder cancer risk in human (Marcus, Hayes, et al., 2000; Marcus, Vineis, & Rothman, 2000) and for GSTA1 and GSTM1 (glutathion S-transferases) for which low activity null-genotypes were found associated with bladder cancer risks in association with smoking (Matic, et al., 2013). The implication of liver enzymes involved in the metabolism of aromatic amines such as the UGT enzymes involved in the O- or N-glucuronidation, and the SULT enzymes involved in sulfatation, have also been hypothesized but need further confirmation. Ethnicity may also have an impact on bladder cancer risk, as several studies evidenced a disparity in disease-specific survival between blacks and whites or other ethnic groups, with a poorer survival for black patients (Yee, Ishill, Lowrance, Herr, & Elkin, 2011). Socioeconomic status, medical conditions (including chronic inflammation, or endemic parasitosis with schistosomes that is observed in Northern part of Africa) or more generally access to care, are some variables that could possibly explain such a difference, though further investigations are needed to validate these hypotheses and reduce this disparity. This is of utmost importance since the management of bladder

cancers represents a real economic burden with a lifetime cost ranging from \$100,000 to \$120,000, the major part of the expenses being associated with surveillance and treatment of recurrences (Avritscher, et al., 2006).

Staging of bladder tumors is performed according to the TNM classification. It includes non muscle-invasive tumors that are classified into three groups: pTa papillary tumors from variable grade and pTis high grade flat tumors, that do not show invasion of the lamina propria and pT1 papillary tumors from variable grades where the lamina propria is invaded. Other tumors are high grade muscle-invasive tumors and are classified as pT2, pT3 or pT4 tumors. More than 90% of tumors are conventional urothelial carcinoma, the other subtype being urothelial carcinoma with aberrant differentiation such as squamous/glandular differentiation, small cell carcinoma, sarcomatoid carcinoma or micropapillary carcinoma (Black, Brown, & Dinney, 2009; Kantor, Hartge, Hoover, & Fraumeni, 1988). At the time of diagnosis 75 to 85% of the tumors are non-muscle invasive tumors, among which 60 to 70% will recur within the first year whereas and only 10 to 20% will progress to muscle-invasive and/or metastatic forms of the disease. Transurethral resection of the bladder represents the treatment of choice for non muscle-invasive tumors. It can be associated with endovesical instillation of mitomycin C in the case of moderate risk of recurrence or of BCG (*Calmette Guérin Bacillus*) in the case of high risk of recurrence or resistance to Mitomycin C (Babjuk, et al., 2013). Cystectomy is used as a curative treatment for non-metastatic forms of muscle-invasive tumors, for non urothelial tumors or tumors that are refractory to bladder-conservatory treatments. It could also be proposed in the case of high risk non muscle-invasive tumors and in some palliative situations (Witjes, et al., 2014). Half of the patients with muscle-invasive tumors (classified as pT2, pT3 or pT4) will develop a metastatic disease within two years. Unfortunately, the median survival of patients with lymph node metastasis is of 18 months and of only 15 months for those with distant metastases (von der Maase, et al., 2005).

### **3.2 Chemotherapies of bladder cancers**

Urothelial bladder cancers are, to some extent, sensitive to chemotherapy with objective response rates reaching 50%. However these tumors cannot be cured and relapse is invariably observed following first line chemotherapy. Unfortunately, no improvement of overall survival could be

achieved within the past 20 years. For locally advanced or metastatic forms of urothelial carcinoma, the best treatment remains cisplatin-based chemotherapies with a median survival of 14 to 15 months for eligible patients (Bamias, Tiliakos, Karali, & Dimopoulos, 2006; von der Maase, et al., 2005). For non-eligible patients, several carboplatin-based chemotherapies have been tested with similar results (Sio, et al., 2014). Other single-agent chemotherapies, or combinations of gemcitabine with these agents, have been the object of multiple phase II clinical trials (C. C. Lin, Hsu, Pu, & Vogelzang, 2008; Sio, et al., 2014). These chemotherapies included the main classes of anticancer agents with drugs such as taxanes (docetaxel, paclitaxel), alkylating agents (ifosfamide, oxaliplatin), antimetabolites (5-FU, pemetrexed), the topoisomerase I inhibitor topotecan, tyrosine kinase inhibitors (lapatinib, sorafenib, gefitinib, sunitinib) or the proteasome inhibitor bortezomib (J. J. Kim, 2012; C. C. Lin, et al., 2008; Sio, et al., 2014). Despite some encouraging response rates as compared to standard chemotherapies, no significant change in overall survival could be noticed (J. J. Kim, 2012; C. C. Lin, et al., 2008; Sio, et al., 2014). A phase III trial with the vinca-alkaloid vinflunine associated with best supportive care showed increased overall survival (6.9 vs 4.3 months) when compared to best supportive care alone (Bellmunt, et al., 2013). Vinflunine is currently the unique drug being approved by EMEA as second line treatment of UC.

As mentioned earlier, new chemotherapies are still under evaluation and results from phase II trials are awaited to define their positioning as compared to already approved regimens (Tables 2, 3 & [www.clinicaltrials.gov](http://www.clinicaltrials.gov)). Concerning taxanes, encouraging tumor response were noticed for the nanoparticle albumin-bound derivative of paclitaxel, abraxane. The phase II trial with cabazitaxel, a derivative that is less susceptible than docetaxel to the efflux pump ABCB1 (formerly called P-glycoprotein), is still ongoing and the phase II trial comparing larotaxel to gemcitabine/cisplatin combination was discontinued prematurely. Clinical trials with EGFR-targeted therapies such as cetuximab or gefitinib were disappointing and responses obtained with VEGF signaling inhibitors bevacizumab or sunitinib, or with the mTOR inhibitor everolimus (Fig. 5) were not better than second line treatment (J. J. Kim, 2012; C. C. Lin, et al., 2008; Sio, et al., 2014). However, in the case of everolimus, a recent report clearly showed that patients with specific alterations of the TSC1 gene displayed significantly prolonged survival (Iyer, et al., 2012), emphasizing the importance of the genetic background of the tumors in the response to chemotherapies, especially therapies that target the PI3K/AKT/mTOR pathway.

### 3.3 Genetic alterations in bladder cancers

Deciphering the genetic background of urothelial cancers has been the object of numerous studies using “standard” approaches such as direct sequencing, CGH or CGH arrays, FISH analyses, or IHC. Despite some variations in the number of patients included in these studies and the disparity in tumor subtypes that were analyzed, it was striking to note the recurrent nature of these alterations, both in terms of chromosome gains or losses, or in terms of point mutations. Losses or deletions were observed for chromosomes 1q, 3p, 4p, 8p, 11p, 9pq, 12p, 13q, 15q, 16q, or 17q, leading to the inactivation of numerous tumor suppressor genes that are present in these loci (Guo, et al., 2013; Iyer, et al., 2012; Lindgren, et al., 2012; Mhawech-Fauceglia, Cheney, & Schwaller, 2006; Network, 2014; Richter, et al., 1999; Simon, et al., 2000). As an example, frequent loss of 3p11 is associated with the inactivation of the fragile histidine triad (FHIT) gene that is frequently observed in late stage bladder cancers and has been associated with poor survival (Skopelitou, Gloustanou, Bai, & Huebner, 2001). Interestingly, inactivation of FHIT by hypermethylation of its promoter was also observed in 16% of bladder tumors (Maruyama, et al., 2001). Deletion of 9pq and 10q are associated with the loss of CDKN2A and PTEN functions, respectively. Similarly, deletion of 13q is associated with the inactivation of the RB1 tumor suppressor gene involved in cell cycle control (Smith, et al., 1999). Loss of RB1 and PTEN are linked with the progression of in situ carcinomas towards more aggressive high grade tumors and with reduced overall survival (Castillo-Martin, Domingo-Domenech, Karni-Schmidt, Matos, & Cordon-Cardo, 2010). In terms of chromosome amplifications, gains of 1q, 3pq, 5p, 6p, 7q, 8pq, 11q, 12q, 17q, and 20q have been observed (Guo, et al., 2013; Iyer, et al., 2012; Mhawech-Fauceglia, et al., 2006; Network, 2014; Prat, et al., 2001; Richter, et al., 1999; Simon, et al., 2000) and are often accompanied by the overexpression of oncogenes, some of them being specifically associated with a subset of aggressive tumors. Among them one could cite MYC, TRIO, oncogenes from the receptor protein tyrosine kinase (RTK) family such as EGFR1, ERBB2, FGFR1 or MET, the cyclins CCND1 and CCNE1 involved in G1/S transition, transcription factors such as PPARG or the RB1-interactant E2F3, the E3-ligase MDM2, genes involved in the methylation of histones such as SETDB1 or genes involved in apoptosis such as BCL2L1 (Goebell & Knowles, 2010; Mhawech-Fauceglia, et al., 2006). Along with chromosome copy number variations, a series of gene rearrangements and activating point mutations have also been identified in genes already known to drive tumor progression such as TP53, FGFR3, RB1, BRAF or HRAS (Lindgren, et al., 2012; Williams,

Hurst, & Knowles, 2013). While TP53 mutations are characteristic from invasive tumors, frequency of FGFR3 amplifications and activating point mutations reaches is more elevated in low grade tumors than in high grade muscle-invasive tumors (80% vs 50%). This suggested the existence of two distinct pathways implicating either FGFR3 or TP53 for tumor development (Goebell & Knowles, 2010; Lindgren, et al., 2012). Activating fusions of FGFR3 with the TACC3 or BAIAP2L1 genes were also observed in some cases and conferred enhanced sensitivity to FGFR inhibitors (Williams, et al., 2013; Wu, et al., 2013).

The PI3K/AKT/mTOR signaling pathway was also found to be altered in a substantial number of bladder tumors (more than 40% of urothelial carcinoma). As it will be detailed further, these alterations correspond to mutations of several key genes regulating this pathway, including the p110 $\alpha$  and the p85 $\alpha$  subunits of PI3K (referred to as PIK3CA and PIK3R1, respectively), AKT, PTEN, TSC1, TSC2, or LKB1 (Table 1).

With the development of new generation sequencing and the use of integrative approaches, a more defined picture of the genomic landscape of urothelial tumors could be obtained by several groups analyzing independent cohorts of patients (Guo, et al., 2013; Iyer, et al., 2012; Lindgren, et al., 2012; Mhawech-Fauceglia, et al., 2006; Network, 2014). Reassuringly, most alterations that were observed in former systematic studies were confirmed and converged towards a classification of bladder cancers that would rely more on the level of alterations and the combination of genes that are altered, rather than on the pathological grading of the tumors (Lindgren, et al., 2012; Network, 2014). These studies also confirmed significant rate of alterations in key genes involved in the RTK-MAPK-RAS-RAF and the PI3K/AKT/mTOR pathways (more than 40% for both pathways), further reinforcing their status of potential “targetable” pathways in the treatment of bladder cancers. Interestingly, these comprehensive studies also identified mutations in genes that were not found to be mutated at a significant level in other tumor types (Network, 2014). For instance, mutations in genes involved in chromatin remodeling such as UTX, MLL-MLL3, CREBBP-EP300, NCOR1, ARID1A or CHD6 genes were detected in more than half of UC patients analyzed (Gui, et al., 2011). The Cancer Genome Atlas Research Network study analyzing a cohort of 131 patients with high grade muscle-invasive urothelial bladder carcinoma, reported recurrent mutations in a series of genes including TP53, FGFR3, NF2, ARID1A, KDM6A, MLL2 (also called KMT2D), CDKN1A, ERCC2, STAG2, RXRA, ELF3, NFE2L2



(formerly called NRF2), KLF5, TXNIP, FOXQ1, RHOB, FOXA1, PAIP1, BTG2, ZFP36L1, RHOA and CCND3 (Gui, et al., 2011; Guo, et al., 2013; Network, 2014; Platt, et al., 2009; Sjobahl, et al., 2011), among which 9 (CDKN1A, ERCC2, RXRA, ELF3, KLF5, FOXQ1, RHOB, PAIP1, BTG2) were not found to be significantly altered in any other types of cancers (Network, 2014). More recently, a truncating mutation associated with inactivation of the STAG2 gene involved in chromosome segregation was identified in 36% of non-invasive bladder cancers that were not prone to recurrences, and in 16% of invasive tumors (Solomon, et al., 2013). Together, these results open new area of research in the identification of potential targetable mutations that could be used for the development of new treatments for urothelial carcinoma of the bladder.

### **3.4 Alterations of the PI3K/AKT/mTOR pathway as targets in urothelial carcinomas**

As mentioned earlier, up to 40% of urothelial carcinomas of the bladder display constitutive activation of the PI3K/AKT/mTOR pathway (Bambury & Rosenberg, 2013; Ching & Hansel, 2010; Knowles, et al., 2009) resulting from either inactivating deletions or mutations of tumor suppressor genes such as PTEN or TSC1, or activating amplifications or mutations of proto-oncogenes such as PIK3CA or AKT1. These alterations are found independently of the urothelial carcinoma subtype, which probably indicates their causal role in tumorigenesis. Table 1 provides an updated detailed inventory of the alterations in the key genes involved in the PI3K/AKT/mTOR pathway that have been reported in bladder tumors from a variety of patient cohorts. Overall, it is remarkable to observe similar mutation rates across studies, despite a heterogeneity in the proportions of patients with non muscle-invasive vs muscle-invasive tumors that were included in these studies and regardless of the technique used to determine these alterations. It is also interesting to note the discrepancy in mutation rates among the different factors implicated in the pathway, as no alteration of mTOR could be observed while PIK3CA, the p110 $\alpha$  subunit of PI3K showed a very high rate of mutations (around 20% for most studies) that is unique to bladder cancers. Mutations of PIK3CA are predominantly located within hotspots such as E542 and E545 residues within the helical domain, and H1047 in the kinase domain of the protein, mutations of other residues being observed at much lower frequency. It is interesting to note that these activating mutations can confer significant advantages in terms of proliferation, cell motility, cell migration, and resistance to anoikis when expressed in normal human urothelial cells (Ross, Askham, & Knowles, 2013). Several mutations in PIK3R1 (the p85 $\alpha$  subunit of PI3K) were also observed (Table

1). These mutations are predominantly located within the BH domain of interaction with PTEN, or in the SH2 domain of the protein that is involved in its interaction with p110 $\alpha$ , indicating they oncogenic role in urothelial carcinoma via the deregulation of p110 $\alpha$  and PTEN functions. In the case of LKB1, a single study reported a missense mutation at serine 19 resulting in early stop codon for approximately 80% of the 50 patients included in the study (Tigli, et al., 2013). It remains to be seen whether this high mutation rate is linked to the specificity of this cohort, or whether this is a recurrent alteration in bladder cancers.

Loss of PTEN or reduced expression of PTEN represents the most frequent alteration that can be found in bladder cancers. Most studies investigating PTEN status reported loss of heterozygosity at significant rates, whereas inactivating point mutations are relatively rare (Table 1). PTEN deficiency is often associated with the activation of the PI3K/AKT/mTOR pathway which explains why PTEN negative tumors showed better responses to mTOR inhibitors, a rationale that have been used to test these derivatives in bladder cancers (Neshat, et al., 2001; Podsypanina, et al., 2001; Shi, et al., 2002; Steelman, Navolanic, et al., 2008). However, one should be cautious about the impact of PTEN loss, as a phase II trial investigating the mTOR inhibitor everolimus in patients with locally advanced or metastatic transitional cell carcinoma of the urothelial tract found an unexpected correlation between PTEN loss and resistance to the drug (Seront, et al., 2012). In fact, PTEN loss was accompanied with AKT activation in response to mTOR inhibition by a negative feedback loop, the mechanism of which remains to be fully elucidated (Seront, et al., 2013). More recently, a retrospective study analyzing PTEN status (among other markers of the PI3K/AKT pathway) in patients with advanced endometrial carcinoma treated by everolimus concluded that expression of proteins of the PI3K/mTOR pathway could not predict response to this mTOR inhibitor (Tredan, et al., 2013). Further studies are clearly needed to investigate the place of PTEN status in response to anticancer drugs targeting the PI3K/AKT/mTOR pathway in urothelial cancers.

AKT1 was also found to be mutated in bladder cancers, at a rate not exceeding 5% (Table 1), with a specific hotspot concerning the E17K substitution in the PH domain of the protein (Carpten, et al., 2007). This mutation results in constitutive activation of AKT1 and its localization to the plasma membrane independently of upstream signaling (Carpten, et al., 2007). It is also mutually exclusive from PIK3CA mutations and PTEN loss. As far as RHEB is concerned, only a single missense

mutation (Y35C) could be detected in one study analyzing 99 tumors of patients with transitional cell carcinoma (Guo, et al., 2013).

As revealed by several studies TSC1 and TSC2 genes that are upstream of mTOR and control its activity via RHEB, were found to be mutated at significant rates in bladder cancers, regardless of the tumor status (Table 1). TSC1 displayed a wide spectrum of mutations including nonsense, missense, frameshift, and splicing mutations, as well as in-frame deletions. These inactivating mutations are distributed throughout the TSC1 gene and may in principle affect all the functions of the corresponding protein (Network, 2014). In general the tumors bearing these mutations also showed a concomitant loss of the wild-type allele (Platt, et al., 2009). LOH was also observed for TSC2, but conversely to TSC1, fewer inactivating point mutations could be observed (Table 1). In patients with TSC2 mutations, no concomitant TSC1 mutations could be observed (Sjodahl, et al., 2011).

#### **4. Drugs targeting the PI3K/AKT/mTOR signaling pathway**

The drugs that are known to target the PI3K/AKT/mTOR pathway are detailed in figures 2-6. They can be classified in five categories depending on their relative specificity towards each target of the pathway. They include the PI3K inhibitors (Fig. 2), the PI3K/mTOR dual inhibitors (Fig. 3), the AKT inhibitors (Fig. 4), the mTOR inhibitors (Fig. 5) and the PDK1 inhibitors (Fig. 6). Table 2 gives a list of all these inhibitors depending on their targets and the actual stage of their development in various types of cancers.

##### **4.1. PI3K inhibitors**

Wortmannin and the quercetin derivative LY-294002 were the first derivatives to be used as PI3K inhibitors, but because of their lack of specificity, they were only used as research tools and were not pushed further into clinic. Many other pan-isoforms PI3K inhibitors with various chemical structures have been identified and are tested in clinical trials (Rodon, Dienstmann, Serra, & Tabernero, 2013). They include a series of wortmannin derivatives such as the pegylated-17-hydroxywortmannin PWT-458 that is more stable, the structural analog PX-866 that irreversibly inhibits PI3Ks (Ihle, et al., 2004) and is in phase II clinical trial for various solid tumors. In phase I, PX-866 showed some partial responses and disease stabilizations when combined with docetaxel, but no significant association

between PIK3CA status and the extent of PFS could be observed (Bowles, et al., 2013). For the peptide derivative of LY-294002, SF1126, in vitro and in vivo preclinical data showed promising activities in the treatment of multiple myelomas (De, et al., 2013). GDC-0941 that inhibits PI3K $\alpha$  and  $\delta$  also targets HIF1 $\alpha$ , has been successfully used in combination with MEK inhibitors for the treatment of non-small cell lung cancers (Zou, et al., 2012). BAY 80-6946 also inhibits both PI3K  $\alpha$  and  $\delta$  and showed peculiar efficiency in tumors with activated PI3K (N. Liu, et al., 2013). NVP-BKM120 (Buparlisib) (Maira, et al., 2012) has been tested in multiple advanced solid tumors (Rodon, et al., 2014), including bladder cancer as second line treatment (Table 3). XL-147 was tested in patients with refractory advanced solid malignancies. As expected, it inhibits the PI3K/AKT/mTOR pathway, but was also found to inhibit the MEK/ERK pathway significantly (Shapiro, et al., 2014). Other derivatives specifically inhibit class I PI3K such as ZSTK474 or CH5132799. Preclinical data showed promising activity in solid tumors for ZSTK474 accompanied with anti-inflammatory effects (Yaguchi, et al., 2006). CH5132799 had also an inhibitory activity towards PI3K mutants in vitro and showed activity in tumors harboring PIK3CA mutations (Tanaka, et al., 2011). Other compounds were reported to specifically target a single isoform of PI3K. For instance, PIK75 (Knight, et al., 2006) and NVP-BYL719 inhibit p110 $\alpha$ , NVP-BYL719 being the object of numerous trials in advanced tumors alone or in combinations with MEK inhibitors (Furet, et al., 2013). GSK-2636771 and TGX221 specifically inhibit p110 $\beta$ . Of particular interest is the specific inhibitor of p110 $\gamma$ , AS-252424, that prevents the growth of T24 bladder cancer cells induced by the stimulation of kinin receptors which is associated with the activation of the PI3K/AKT pathway (Sgnaolin, et al., 2013). AS-252424 is thus representing an attractive option for the treatment of bladder cancers with constitutive activation of PI3K. Finally, the IC87114 compound and its oral form derivative CAL-101 specifically target PI3K $\delta$ , an isoform that is specifically expressed in lymphocytes, providing a special interest to develop these derivatives in the treatment of hematological malignancies. CAL-101 inhibits the PI3K signaling by preventing AKT1 phosphorylation at threonine 308 (Lannutti, et al., 2011) and provided approximately 30% partial responses in chronic lymphocytic leukemia. Recently, the characterization of GS-9820 as a new selective inhibitor of PI3K $\delta$  emphasized the role of this PI3K isoform in the regulation of osteoclast morphology, actin cytoskeletal organization, and resorptive activity (Shugg, et al., 2013).

## 4.2. Dual PI3K-mTOR inhibitors

The similarity between mTOR and PI3K ATP catalytic site has rendered possible to design compounds that could inhibit both kinases (including both mTORC1 and mTORC2 complexes), resulting in higher efficiency than rapamycin analogs alone, a phenomenon that could be explained by the negative feed-back function of mTOR, the inhibition of which can activate PI3K via S6K and the RAS/RAF/MEK/ERK pathway (Fig. 1) (Engelman, 2009). Therefore, inhibiting both mTOR and PI3K could prevent such a feed-back loop and enhance treatment efficacy. In this line, combination with MEK inhibitors could be of interest, though it is not known whether cumulative toxicities could be tolerable. Another interesting feature resides in the fact that dual inhibitors could also block the proliferation of cancer cells with PIK3CA activating mutations (Brachmann, Fritsch, Maira, & Garcia-Echeverria, 2009). The first dual PI3K/mTOR inhibitor to be described is PI-103. It belongs to the pyridofuopyrimidine family and inhibits class I PI3K at nanomolar ranges and mTOR at sub-micromolar concentrations (Raynaud, et al., 2007). PI-103 was active against a wide range of cancer cell lines and induced tumor delay in xenograft models despite an extensive metabolization (Raynaud, et al., 2007). PI-103 showed promising activity in AML and in glioblastoma and could potentiate the effects of radiotherapy and chemotherapy (Prevo, et al., 2008; Westhoff, et al., 2009). The PI-103 derivative WJD008 shows enhanced inhibitory activity towards p110 $\alpha$  and mTOR and was found to suppress the growth of transformed cells harboring PIK3CA activating mutations (Ponzo & Park, 2010). The imidazoquinoline derivative NVP-BEZ235 inhibits all forms of PI3K and was active in several preclinical tumor models including breast cancer cells expressing the PIK3CA activating mutation H1047R (Serra, et al., 2008). NVP-BEZ235 shows synergistic or additive effects with several cytotoxic agents in cancer cell lines or in xenograft models (Baumann, Mandl-Weber, Oduncu, & Schmidmaier, 2009; Manara, et al., 2010). Interestingly, NVP-BEZ235 and the new derivative NVP-BTG226 also enhanced radiosensitivity in cancer cell lines including the HRAS mutated T24 bladder cancer cell line (Fokas, et al., 2012). Open label phase I with NVP-BEZ235 showed activity in patients with activated PI3K despite a need for pharmacokinetic improvement (H. Burris, et al., 2010). Three morpholino 1,3,5-triazine PKI compounds, PKI-402, PKI-587 (also known as PF-0521238) and its orally available derivative PKI-179 also showed potent inhibitory activity against class I PI3K (including

mutant PIK3CA) and mTOR (Mallon, et al., 2010; Venkatesan, et al., 2010; Yuan, et al., 2011). These derivatives are active in various tumor cell models in vitro and in vivo and the results of clinical trials are awaited. PF-04691502 is an orally available dual PI3K/mTOR inhibitor that showed potent inhibition of PIK3CA-mutant and PTEN-null cell lines proliferation (Yuan, et al., 2011). Results of phase I study demonstrated PF-04691502 efficacy to decrease the phosphorylation of downstream effectors of PI3K in tumor tissue (Britten, et al., 2014). XL-765 is a dual inhibitor that prevents AKT phosphorylation induced by PI3K/mTORC2 (Prasad, et al., 2011). XL-765 has been developed as monotherapy or in combination with other chemotherapy for the treatment of a broad range of malignancies and is the object of many ongoing clinical trials. The morpholino thienopyrimidine derivatives GDC-0980 and its analog GNE-477 (Heffron, et al., 2010) represent another class of dual PI3K/mTOR inhibitors that are active in activated PI3K cancer cell models (Wallin, et al., 2011). Clinical trials are currently ongoing in advanced solid tumors and Non-Hodgkin's lymphoma. Similarly the two dual inhibitors GSK-2126458 and GSK-1059615 are in clinical trials for advanced solid tumors and lymphoma.

#### **4.3. AKT inhibitors**

Few AKT inhibitors have been developed so far, probably because of the secondary effects linked to the multiple regulatory functions of AKT (Table 1). The alkylphosphocholine perifosine (KRX-0401) targets the pleckstrin homology domain of AKT, leading to the inhibition of PDK1-dependent activation of AKT and the inhibition of its translocation to the cell membrane (Krawczyk, et al., 2013). It is active in numerous cancer lines and potentiates radiotherapy and cell response to various chemotherapies. Perifosine has been the object of extensive number of clinical trials alone or in combination but the results of two phase III trials in colorectal cancer and multiple myeloma failed to show efficacy. The MK-2206 derivative is an allosteric inhibitor that also targets the pleckstrin homology domain of AKT. It is particularly active in a subset of colorectal cancer cells that are dependent on IGF-1R and was recently shown to exert apoptotic effects via two distinct mechanisms: the induction of apoptosis inducing factor (AIF), and the inhibition of the cytoskeletal protein Ezrin (Agarwal, et al., 2014). MK-2206 is being tested alone or in combination in many types of solid tumors and hematological malignancies and provided some encouraging antitumoral activity. The mechanism of AKT inhibition by Triciribine (API-2) is not well defined, but preclinical studies clearly demonstrate its capability to inhibit the phosphorylation of all AKT members and the growth of tumor xenografts

models overexpressing AKT (Mirzoeva, et al., 2011). GSK-690693 is an ATP-competitive specific inhibitor of AKT members, and has shown significant activity in various tumor models (Rhodes, et al., 2008), but the clinical trials were terminated. GDC-0068 is a very selective ATP-competitive inhibitor of all AKT members. It showed robust anticancer activity in vitro and in xenograft models, and this activity was correlated with various markers of AKT activation (J. Lin, et al., 2013). Furthermore, GDC-0068 enhanced cell response to other classical anticancer agents (J. Lin, et al., 2013). KP372-1 is an equal mixture of two isomers that inhibit AKT, PDK1 and FLT3 (Zeng, et al., 2006). By inhibiting the AKT downstream targets and the FLT3/PIM signaling, these small molecules were efficiently inhibiting AML cell growth while barely affecting normal hematopoietic cells (Zeng, et al., 2006). A-443654 is an indazole-pyridine based highly selective AKT inhibitor with a narrow therapeutic window (Luo, et al., 2005) that exerts its cytotoxicity by interfering with mitotic progression and bipolar spindle formation via the inhibition of the promoter activity of Aurora Kinase (X. Liu, et al., 2008). AZD-5363 inhibits all three isoforms of AKTs and P70S6K at the same concentration. It inhibits the growth of a wide range of cancer cell lines and sensitivity to the drug was correlated with the presence of activating PIK3CA mutations, or with PTEN loss or inactivating mutation (Davies, et al., 2012). AZD5363 is active in tumor xenografts, as monotherapy or in combination with docetaxel and has entered several clinical trials for solid malignancies (Davies, et al., 2012). XL-418 was shown to inhibit both AKT and S6K but the only clinical trial in solid tumors with this molecule was suspended.

#### **4.4. mTOR inhibitors**

Rapamycin and its analogs (rapalogs) were the first selective mTOR inhibitors to enter into the clinic. They act as allosteric inhibitors of mTORC1 via their association to the FKBP12 protein, resulting in the dissociation of the complex and subsequent inhibition of mTOR signaling (Lamming, Ye, Sabatini, & Baur, 2013). Everolimus and temsirolimus are approved by the FDA for the treatment of several cancers including renal cell carcinoma, lymphoma, astrocytoma and pancreatic cancers (Benjamin, Colombi, Moroni, & Hall, 2011). The ridaforolimus is in clinical trial for the treatment of sarcomas (Chawla, et al., 2012). It is interesting to note that sensitivity to rapalogs was associated with the presence of activating PIK3CA mutations, or with PTEN inactivation due to PTEN loss or the presence of missense mutations (Carew, Kelly, & Nawrocki, 2011). Activity of rapalogs is also probably linked to their antiangiogenic effects as treatment of renal cancer cells induced a marked reduction of HIF-1 $\alpha$  levels (Mahalingam, et al., 2010).

Besides rapalogs, a variety of other mTOR inhibitors have been developed and can target both mTORC1 and mTORC2 complexes. This is the case for PP-242 and its derivative INK-128 that are potent and selective ATP competitor of both complexes and have shown antitumoral activity against a variety of cancer types. It is surmised that the greater effect of PP-242 over rapamycin in multiple myeloma cell lines could be attributed to the inhibition of mTORC2 (Hoang, et al., 2010). INK-128 has entered clinical trials for advanced solid malignancies and multiple myelomas. OSI-027 and OXA-01 are other selective inhibitors of mTORC1 and mTORC2 complexes and inhibit the phosphorylation of Akt and of several downstream targets of mTORC1 such as S6K, S6 riboprotein, and 4EBP1 (Bhagwat, et al., 2011; Falcon, et al., 2011). Preclinical data demonstrated that treatment with OSI-027 reduced cell proliferation in various cancer cell lines, including bladder cancer cells in which association with lapatinib was highly synergistic (Becker, et al., 2013; Bhagwat, et al., 2011). Treatment with OXA-01 also showed significant anti-angiogenic effects and slowed vessel regrowth after discontinuation of VEGFR inhibition (Falcon, et al., 2011). A phase I with OSI-027 in lymphoma or advanced solid tumors has been completed and showed some antitumoral activity (Tan, et al., 2010).

Palomid 529 is a pan mTOR inhibitor with strong antitumor effects associated with potent antiangiogenic effects, but is only tested in patients with age-related macular degeneration. The Ku0063794 derivative is a highly selective inhibitor of mTORC complexes and inhibited downstream effectors of mTOR such as AKT and S6 (Pike, et al., 2013). The relatively low potency of Ku0063794 in cell models led to its optimization and the identification of the clinical derivatives AZD-8055 and AZD-2014 (Pike, et al., 2013). These compounds retained a high selectivity towards mTOR but showed improved aqueous solubility and cellular potency. The AZD-2014 was developed from AZD-8055 in order to improve the pharmacokinetic parameters of the compound especially to reduce its turnover in human hepatocytes (Pike, et al., 2013). AZD-2014 shows a potent activity towards colon cancer cells in vitro and in vivo and seems to induce an autophagic death rather than apoptosis (Huo, et al., 2014). Both compounds are currently in clinical trials for advanced solid malignancies.

A series of pyrazolopyrimidine, WAY-600, WYE-132, WYE-687, and WYE-354 have also been tested and specifically inhibited both mTORC complexes at nanomolar ranges in vitro, a selectivity that was retained in various cancer cell lines (Yu, et al., 2010; Yu, et al., 2009). These compounds were also active in xenograft models and may enter clinical trials.



Torin 1 is a pyridinonequinoline derivative isolated from a screen to identify selective mTOR inhibitors (Thoreen, et al., 2009). Torin1 exerts its activity by interacting with the unique Trp2239 residue of mTOR within the catalytic site of the kinase (Yang, et al., 2013). Torin2 is an orally available derivative of Torin1 with improved metabolic stability and plasma exposure (Q. Liu, et al., 2011). Torin2 inhibits mTORC1-dependent phosphorylation of S6K but, unlike Torin1, also targets the PIKKs ATM, ATR and DNA-PK, sensitizing cells to ionizing radiations (Q. Liu, et al., 2013). Torin2 displayed a potent activity against a panel of lung cancer cell lines inducing both apoptosis and autophagy, and showed synergistic activity in combination with a MEK inhibitor (Q. Liu, et al., 2013).

#### **4.5. PDK1 inhibitors**

NVP-BAG956 is an imidazoquinoline derivative which targets PDK1 as well as PI3K. As compared to all other classes of inhibitors of the mTOR pathway, NVP-BAG956 was the most potent agent against T-ALL cell lines and potentiated the antileukemic activity of both nilotinib and RAD001 in vivo (Weisberg, et al., 2008). OSU-03012 (AR-12) was synthesized based on the celecoxib backbone, but does not retain the inhibitory effect on COX2 (Zhu, et al., 2004). Though OSU-03012 can inhibit PDK1 and AKT signaling at specific concentrations, various studies suggested that activation of ER-stress proteins and modulation of HSP70 and HSP90 chaperones could also participate to its cellular toxicity in various cancer types. OSU-03012 showed synergistic effects with lapatinib in glioma and breast cancer cells (Booth, et al., 2012; West, Garcia-Vargas, Chalfant, & Park, 2013). OSU-03012 is in clinical trial for lymphoma and solid tumors.

BX-912, BX-320, and BX-795 were synthesized from an amidopyrimidine backbone and identified as PDK1 selective inhibitors that could block the PDK1 activity in tumor cells and induce cell growth inhibition and/or apoptosis in vitro, BX-320 being also active in in vivo tumor models (Feldman, et al., 2005). More recently, it was shown that BX-795 could also achieve a G2 block in PDK1<sup>-/-</sup> cells, suggesting that other targets could be responsible for its cellular toxicity (Tamguney, Zhang, Fiedler, Shokat, & Stokoe, 2008). Indeed BX-795 also inhibits Cdk1, Cdk2, and Aurora A, B and C with similar potencies (Tamguney, et al., 2008). Nevertheless, these BX derivatives represent interesting optional treatments to be tested in the clinic. As compared to other inhibitors described above, the small molecule GSK-2334470 displayed a much higher selectivity towards PDK1 and showed a potent

cytotoxicity towards glioblastoma and renal cancer cell lines (Najafov, Sommer, Axten, Deyoung, & Alessi, 2011).

### **5.The use of PI3K/AKT/mTOR inhibitors to treat bladder cancers**

As already mentioned earlier, the plethora of genomic alterations of the PI3K/AKT/mTOR signaling pathway that were identified in bladder tumor samples emphasized the importance of this pathway in the onset and/or the progression of urothelial carcinoma, pointing towards its use as a potential target. Preclinical studies showed the efficacy of rapamycin in inhibiting the growth of bladder cancer cells in vitro (Fechner, Classen, Schmidt, Hauser, & Muller, 2009). Similar results were also observed for everolimus (RAD001) that could significantly inhibit the growth of nine bladder tumor cell line (Mansure, et al., 2009), and in xenograft models, an effect that was accompanied with inhibition of the downstream effectors of mTOR such as inhibition of protein synthesis through the S6K and 4EBP1 pathways, and reduction in angiogenesis(Chiong, et al., 2011; Mansure, et al., 2009). These data represented a strong biological rationale to use inhibitors of the PI3K-AKT-mTOR pathway in bladder cancers. However, only few clinical trials have been performed, probably because of the low number of patients with metastatic disease and the relative fragility of these patients. mTOR inhibitors everolimus and temsirolimus that were approved in renal and breast cancers have been tested in small series of patients with advanced bladder cancers. These trials were performed in heavily pre-treated patients who were refractory to platinum salts. The results were globally disappointing despite some long-term responses for several patients (Table 3). In one phase II trial, disease control was maintained up to 14 months and was even observed for patients with poor prognostic factors (Seront, et al., 2012). Interestingly, more than half of non-responder patients showed no expression of PTEN and PTEN loss was only observed in non-responders patients, emphasizing the potential role of PTEN as a biomarker of tumor response to everolimus. Similar results were obtained in the other phase II trial with a median progression free survival of 2.6 months and a median overall survival of 8.3 months (Milowsky, et al., 2013). In order to investigate the molecular basis for specific responses to everolimus, whole genome sequencing of 14 patients included in the phase II trial of Seront et al. were performed retrospectively and evidenced an inactivating mutation of the TSC1 gene (Iyer, et al.,

2012). Further analysis of 5 patients with significant responses to everolimus revealed that 4 patients were also mutated for TSC1, response rate being different depending on the mutation (Iyer, et al., 2012). This discovery further reinforced the importance of the PI3K/AKT/mTOR signaling pathway as a potential attractive target for bladder cancer, and positioned everolimus as an interesting option for patients with inactivated TSC1. However, it is important to keep in mind that tumors with TSC1 mutations presented different genetic backgrounds that could also explain the differences in patients' responses to everolimus, in particular mutations that were identified at significant rates as mentioned in section 3.3 (Gui, et al., 2011; Guo, et al., 2013; Network, 2014; Platt, et al., 2009; Sjobahl, et al., 2011). To cite one example, activating mutations of NFE2L2, a transcription factor that regulates the anti-oxidant program in response to oxidative stress (Shibata, et al., 2008) was associated with enhanced expression of genes involved in ROS-induced detoxification that could in turn have an impact on everolimus response as mTOR pathway is also involved in the regulation of oxidative stress (Darzynkiewicz, et al., 2014). In this line, alterations leading to activating or inactivating mutations of other genes that were significantly altered in bladder cancer may also influence tumor response to everolimus or other mTOR inhibitors. Temsirolimus was also tested in a phase II trial including 15 patients but, conversely to other trials, no significant responses has been observed, leading to the conclusion that temsirolimus seemed to have poor activity in metastatic transitional carcinoma of the urothelium that are resistant to platinum (Gerullis, et al., 2012). Other clinical trials targeting the PI3K/AKT/mTOR signaling pathway have been performed or are currently in progress (Table 3, <http://clinicaltrials.gov>). Paradoxically, these trials are mainly testing associations of mTOR inhibitors everolimus (and temsirolimus for a single trial) with existing cytotoxic agents such as cisplatin, gemcitabine, or paclitaxel. Most of these trials are in the recruiting phase. As of today, we could inventory only two trials with molecules targeting other factors of the PI3K/AKT/mTOR pathway than mTOR alone (Table 3, <http://clinicaltrials.gov>). One phase I trial evaluated the PI3K/mTOR dual inhibitors GSK2126458 in patients with advanced cancers. Interestingly, in patients with bladder cancers objective responses were observed in 1 out of 3 patients with PIK3CA mutations and in two patients out of 15 patients with wild-type PIK3CA (Munster, et al., 2012). From these preliminary results, and the complex genetic landscape of bladder tumors that has recently been revealed by several independent studies on large cohorts using integrative approaches, it seems reasonable to postulate that the activity of PI3K inhibitors does not solely rely on PIK3CA status, but that

Other alterations might also contribute to tumor response including the anti-angiogenic effects of these molecules. The second trial is a phase II trial (NCT01551030) investigating the specific PI3K inhibitor buparlisib (BKM-120) in metastatic transitional cell carcinoma of the urothelium. This trial is currently in its recruiting phase and results are awaited eagerly.

## **6. Conclusions**

The recent studies characterizing the genetic background of urothelial cancers of the bladder, revealed the unicity of this model with alterations that are not seen at the same level in other types of cancers. This is especially the case for the PI3K/AKT/mTOR signaling pathway that occupies a major place in the etiology of these tumors due to its constitutive activation that was observed in a significant number of these tumors. Though no mutations of mTOR itself could be detected, mTOR inhibitors were the first inhibitors of this pathway to be used for the treatment of advanced refractory bladder cancers. However, it appeared that targeting mTOR was only beneficial for a subset of bladder cancer patients, because of negative feed-back loop mechanisms leading to further activation of PI3K. In this line, combinations of mTORC inhibitors with PI3K inhibitors or the use of dual PI3K/mTOR inhibitors may increase the number of effective responses. A pivotal study also demonstrated that other alterations of this pathway such as TSC1 mutations in the case of everolimus, may also play a crucial role in drug response to mTOR inhibitors, further reinforcing the notion that a global genetic mapping of the pathway should be taken into account to assess more precisely the potential clinical benefit of such targeted therapies. Even though a wide range of new specific inhibitors of PI3K, AKT or PDK1 have been characterized at the preclinical level for their potent anticancer activity in vitro or in xenograft models, it is paradoxical to note that only two new inhibitors, a PI3K inhibitor and a dual PI3K/mTOR inhibitor are currently in clinical trial for bladder cancers. This could certainly be explained by the low frequency of this cancer and/or the relative fragility of these patients heavily treated patients, especially those who are refractory to cisplatin-based regimen. An additional level of complexity will have to be taken into account, as regulators of other key cellular pathways such as TP53, FGFR3, RAS, RAF, MEK, or ERK, were found to be altered in a significant number of urothelial tumors and could thus also contribute to tumor response to chemotherapies. In this line, combinations of inhibitors of the PI3K/AKT/mTOR inhibitors with inhibitors of FGFR3 such as dovitinib, or with MEK inhibitors,

may certainly represent alternative options to improve tumor control. Similar reasoning could be performed in the case of combinations with antiangiogenic derivatives that showed promising synergistic effects. With the future of personalized medicine, it is of utmost importance to further characterize at the molecular level each subtype of urothelial cancers in order to identify the best treatment options. The recent identification of significant rates of alterations in genes involved in chromatin remodeling and of epigenetic changes in advanced bladder cancers is another compelling evidence of the genetic complexity of these tumors, but opens a new area of research to identify new derivatives that could specifically target these tumor subtypes. These data emphasize the importance to carefully choose the most pertinent target(s) and the drugs or (drug combinations) to be further tested in the clinic and the necessity of cooperative efforts to conduct these trials.

## References

Agarwal, E., Chaudhuri, A., Leiphrakpam, P. D., Haferbier, K. L., Brattain, M. G., & Chowdhury, S. (2014). Akt inhibitor MK-2206 promotes anti-tumor activity and cell death by modulation of AIF and Ezrin in colorectal cancer. *BMC Cancer*, 14, 1471-2407.

Alessi, D. R., James, S. R., Downes, C. P., Holmes, A. B., Gaffney, P. R., Reese, C. B., & Cohen, P. (1997). Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Balpha. *Curr Biol.*, 7, 261-269.

Asahina, H., Nokihara, H., Yamamoto, N., Yamada, Y., Tamura, Y., Honda, K., Seki, Y., Tanabe, Y., Shimada, H., Shi, X., & Tamura, T. (2013). Safety and tolerability of AZD8055 in Japanese patients with advanced solid tumors; a dose-finding phase I study. *Invest New Drugs*, 31, 677-684.

Askham, J. M., Platt, F., Chambers, P. A., Snowden, H., Taylor, C. F., & Knowles, M. A. (2010). AKT1 mutations in bladder cancer: identification of a novel oncogenic mutation that can co-operate with E17K. *Oncogene*, 29, 150-155.

Aveyard, J. S., Skilleter, A., Habuchi, T., & Knowles, M. A. (1999). Somatic mutation of PTEN in bladder carcinoma. *Br J Cancer.*, 80, 904-908.

Avritscher, E. B., Cooksley, C. D., Grossman, H. B., Sabichi, A. L., Hamblin, L., Dinney, C. P., & Elting, L. S. (2006). Clinical model of lifetime cost of treating bladder cancer and associated complications. *Urology.*, 68, 549-553. Epub 2006 Sep 2018.

Babjuk, M., Burger, M., Zigeuner, R., Shariat, S. F., van Rhijn, B. W., Comperat, E., Sylvester, R. J., Kaasinen, E., Bohle, A., Palou Redorta, J., & Roupret, M. (2013). EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2013. *Eur Urol*, 64, 639-653.

Bambury, R. M., & Rosenberg, J. E. (2013). Actionable mutations in muscle-invasive bladder cancer. *Curr Opin Urol*, 23, 472-478.

Bamias, A., Tiliakos, I., Karali, M. D., & Dimopoulos, M. A. (2006). Systemic chemotherapy in inoperable or metastatic bladder cancer. *Ann Oncol.*, 17, 553-561. Epub 2005 Nov 2022.

Bartholomeusz, C., & Gonzalez-Angulo, A. M. (2012). Targeting the PI3K signaling pathway in cancer therapy. *Expert Opin Ther Targets*, 16, 121-130.

Baumann, P., Mandl-Weber, S., Oduncu, F., & Schmidmaier, R. (2009). The novel orally bioavailable inhibitor of phosphoinositol-3-kinase and mammalian target of rapamycin, NVP-BEZ235, inhibits growth and proliferation in multiple myeloma. *Exp Cell Res.*, 315, 485-497. doi: 410.1016/j.yexcr.2008.1011.1007. Epub 2008 Nov 1027.

Becker, M. N., Wu, K. J., Marlow, L. A., Kreinest, P. A., Vonroemeling, C. A., Copland, J. A., & Williams, C. R. (2013). The combination of an mTORc1/TORc2 inhibitor with lapatinib is synergistic in bladder cancer in vitro. *Urol Oncol*, 2013, 00251-00252.

Bellmunt, J., Fougeray, R., Rosenberg, J. E., von der Maase, H., Schutz, F. A., Salhi, Y., Culine, S., & Choueiri, T. K. (2013). Long-term survival results of a randomized phase III trial of vinflunine plus best supportive care versus best supportive care alone in advanced urothelial carcinoma patients after failure of platinum-based chemotherapy. *Ann Oncol*, 24, 1466-1472.

Benjamin, D., Colombi, M., Moroni, C., & Hall, M. N. (2011). Rapamycin passes the torch: a new generation of mTOR inhibitors. *Nat Rev Drug Discov*, 10, 868-880.

Bertram, J. S., & Craig, A. W. (1972). Specific induction of bladder cancer in mice by butyl-(4-hydroxybutyl)-nitrosamine and the effects of hormonal modifications on the sex difference in response. *Eur J Cancer.*, 8, 587-594.

Bhagwat, S. V., Gokhale, P. C., Crew, A. P., Cooke, A., Yao, Y., Mantis, C., Kahler, J., Workman, J., Bittner, M., Dudkin, L., Epstein, D. M., Gibson, N. W., Wild, R., Arnold, L. D., Houghton, P. J., & Pachter, J. A. (2011). Preclinical characterization of OSI-027, a potent and selective inhibitor of mTORC1 and mTORC2: distinct from rapamycin. *Mol Cancer Ther*, 10, 1394-1406.

Bitting, R. L., & Armstrong, A. J. (2013). Targeting the PI3K/Akt/mTOR pathway in castration-resistant prostate cancer. *Endocr Relat Cancer*, 20, 12-0394.

Black, P. C., Brown, G. A., & Dinney, C. P. (2009). The impact of variant histology on the outcome of bladder cancer treated with curative intent. *Urol Oncol.*, 27, 3-7. doi: 10.1016/j.urolonc.2007.1007.1010. Epub 2008 Jan 1014.

Bogusz, A. M., Brickley, D. R., Pew, T., & Conzen, S. D. (2006). A novel N-terminal hydrophobic motif mediates constitutive degradation of serum- and glucocorticoid-induced kinase-1 by the ubiquitin-proteasome pathway. *Febs J.*, 273, 2913-2928.

Booth, L., Cruickshanks, N., Ridder, T., Chen, C. S., Grant, S., & Dent, P. (2012). OSU-03012 interacts with lapatinib to kill brain cancer cells. *Cancer Biol Ther*, 13, 1501-1511.

Bowles, D. W., Ma, W. W., Senzer, N., Brahmer, J. R., Adjei, A. A., Davies, M., Lazar, A. J., Vo, A., Peterson, S., Walker, L., Hausman, D., Rudin, C. M., & Jimeno, A. (2013). A multicenter phase 1 study of PX-866 in combination with docetaxel in patients with advanced solid tumours. *Br J Cancer*, 109, 1085-1092.

Brachmann, S., Fritsch, C., Maira, S. M., & Garcia-Echeverria, C. (2009). PI3K and mTOR inhibitors: a new generation of targeted anticancer agents. *Curr Opin Cell Biol.*, 21, 194-198. doi: 110.1016/j.ceb.2008.1012.1011. Epub 2009 Feb 1017.

Britten, C. D., Adjei, A. A., Millham, R., Houk, B. E., Borzillo, G., Pierce, K., Wainberg, Z. A., & Lorusso, P. M. (2014). Phase I study of PF-04691502, a small-molecule, oral, dual inhibitor of PI3K and mTOR, in patients with advanced cancer. *Invest New Drugs*, 2014, 7.

Burger, M., Catto, J. W., Dalbagni, G., Grossman, H. B., Herr, H., Karakiewicz, P., Kassouf, W., Kiemeny, L. A., La Vecchia, C., Shariat, S., & Lotan, Y. (2013). Epidemiology and risk factors of urothelial bladder cancer. *Eur Urol*, 63, 234-241.

Burriss, H., Rodon, J., Sharma, S., Herbst, R. S., Taberner, J., Infante, J. R., Silva, A., Demanse, D., Hackl, W., & J., B. (2010). First-in-human phase I study of the oral PI3K inhibitor BEZ235 in patients (pts) with advanced solid tumors. *J Clin Oncol*, 28, suppl; abstr 3005.

Burriss, H. A., 3rd. (2013). Overcoming acquired resistance to anticancer therapy: focus on the PI3K/AKT/mTOR pathway. *Cancer Chemother Pharmacol*, 71, 829-842.

Cairns, P., Evron, E., Okami, K., Halachmi, N., Esteller, M., Herman, J. G., Bose, S., Wang, S. I., Parsons, R., & Sidransky, D. (1998). Point mutation and homozygous deletion of PTEN/MMAC1 in primary bladder cancers. *Oncogene.*, 16, 3215-3218.



Calderaro, J., Rebouissou, S., de Koning, L., Masmoudi, A., Herault, A., Dubois, T., Maille, P., Soyeux, P., Sibony, M., de la Taille, A., Vordos, D., Lebret, T., Radvanyi, F., & Allory, Y. (2013). PI3K/AKT pathway activation in bladder carcinogenesis. *Int J Cancer*, *134*, 1776-1784.

Campone, M., Bachelot, T., Gnant, M., Deleu, I., Rugo, H. S., Pistilli, B., Noguchi, S., Shtivelband, M., Pritchard, K. I., Provencher, L., Burris, H. A., 3rd, Hart, L., Melichar, B., Hortobagyi, G. N., Arena, F., Baselga, J., Panneerselvam, A., Henriquez, A., El-Hashimyt, M., Taran, T., Sahmoud, T., & Piccart, M. (2013). Effect of visceral metastases on the efficacy and safety of everolimus in postmenopausal women with advanced breast cancer: subgroup analysis from the BOLERO-2 study. *Eur J Cancer*, *49*, 2621-2632.

Cantley, L. C. (2002). The phosphoinositide 3-kinase pathway. *Science.*, *296*, 1655-1657.

Cappellen, D., Gil Diez de Medina, S., Chopin, D., Thiery, J. P., & Radvanyi, F. (1997). Frequent loss of heterozygosity on chromosome 10q in muscle-invasive transitional cell carcinomas of the bladder. *Oncogene.*, *14*, 3059-3066.

Carayol, N., Vakana, E., Sassano, A., Kaur, S., Goussetis, D. J., Glaser, H., Druker, B. J., Donato, N. J., Altman, J. K., Barr, S., & Plataniias, L. C. (2010). Critical roles for mTORC2- and rapamycin-insensitive mTORC1-complexes in growth and survival of BCR-ABL-expressing leukemic cells. *Proc Natl Acad Sci U S A*, *107*, 12469-12474.

Carew, J. S., Kelly, K. R., & Nawrocki, S. T. (2011). Mechanisms of mTOR inhibitor resistance in cancer therapy. *Target Oncol*, *6*, 17-27.

Carpenter, C. L., Auger, K. R., Chanudhuri, M., Yoakim, M., Schaffhausen, B., Shoelson, S., & Cantley, L. C. (1993). Phosphoinositide 3-kinase is activated by phosphopeptides that bind to the SH2 domains of the 85-kDa subunit. *J Biol Chem.*, *268*, 9478-9483.

Carpenter, J. D., Faber, A. L., Horn, C., Donoho, G. P., Briggs, S. L., Robbins, C. M., Hostetter, G., Boguslawski, S., Moses, T. Y., Savage, S., Uhlik, M., Lin, A., Du, J., Qian, Y. W., Zeckner, D. J., Tucker-Kellogg, G., Touchman, J., Patel, K., Mousses, S., Bittner, M., Schevitz, R., Lai, M. H., Blanchard, K. L., & Thomas, J. E. (2007). A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. *Nature.*, *448*, 439-444. Epub 2007 Jul 2004.

Carracedo, A., Baselga, J., & Pandolfi, P. P. (2008). Deconstructing feedback-signaling networks to improve anticancer therapy with mTORC1 inhibitors. *Cell Cycle*, 7, 3805-3809. Epub 2008 Dec 3822.

Carracedo, A., Ma, L., Teruya-Feldstein, J., Rojo, F., Salmena, L., Alimonti, A., Egia, A., Sasaki, A. T., Thomas, G., Kozma, S. C., Papa, A., Nardella, C., Cantley, L. C., Baselga, J., & Pandolfi, P. P. (2008). Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. *J Clin Invest.*, 118, 3065-3074. doi: 3010.1172/JCI34739.

Castillo-Martin, M., Domingo-Domenech, J., Karni-Schmidt, O., Matos, T., & Cordon-Cardo, C. (2010). Molecular pathways of urothelial development and bladder tumorigenesis. *Urol Oncol*, 28, 401-408.

Chalhoub, N., & Baker, S. J. (2009). PTEN and the PI3-kinase pathway in cancer. *Annu Rev Pathol.*, 4:127-50., 10.1146/annurev.pathol.1144.110807.092311.

Chang, F., Lee, J. T., Navolanic, P. M., Steelman, L. S., Shelton, J. G., Blalock, W. L., Franklin, R. A., & McCubrey, J. A. (2003). Involvement of PI3K/Akt pathway in cell cycle progression, apoptosis, and neoplastic transformation: a target for cancer chemotherapy. *Leukemia.*, 17, 590-603.

Chawla, S. P., Staddon, A. P., Baker, L. H., Schuetze, S. M., Tolcher, A. W., D'Amato, G. Z., Blay, J. Y., Mita, M. M., Sankhala, K. K., Berk, L., Rivera, V. M., Clackson, T., Loewy, J. W., Haluska, F. G., & Demetri, G. D. (2012). Phase II study of the mammalian target of rapamycin inhibitor ridaforolimus in patients with advanced bone and soft tissue sarcomas. *J Clin Oncol*, 30, 78-84.

Ching, C. B., & Hansel, D. E. (2010). Expanding therapeutic targets in bladder cancer: the PI3K/Akt/mTOR pathway. *Lab Invest*, 90, 1406-1414.

Chiong, E., Lee, I. L., Dadbin, A., Sabichi, A. L., Harris, L., Urbauer, D., McConkey, D. J., Dickstein, R. J., Cheng, T., & Grossman, H. B. (2011). Effects of mTOR inhibitor everolimus (RAD001) on bladder cancer cells. *Clin Cancer Res*, 17, 2863-2873.

Chow, L. W., Tung, S. Y., Ng, T. Y., Im, S. A., Lee, M. H., Yip, A. Y., Toi, M., & Gluck, S. (2013). Concurrent celecoxib with 5-fluorouracil/epirubicin/cyclophosphamide followed by docetaxel for stages II - III invasive breast cancer: the OOTR-N001 study. *Expert Opin Investig Drugs*, 22, 299-307.

Cordes, I., Kluth, M., Zygis, D., Rink, M., Chun, F., Eichelberg, C., Dahlem, R., Fisch, M., Hoppner, W., Wagner, W., Doh, O., Terracciano, L., Simon, R., Wilczak, W., Sauter, G., & Minner, S. (2013).

PTEN deletions are related to disease progression and unfavourable prognosis in early bladder cancer. *Histopathology*, 63, 670-677.

Courtney, K. D., Corcoran, R. B., & Engelman, J. A. (2010). The PI3K pathway as drug target in human cancer. *J Clin Oncol*, 28, 1075-1083.

Crino, P. B., Nathanson, K. L., & Henske, E. P. (2006). The tuberous sclerosis complex. *N Engl J Med.*, 355, 1345-1356.

Dan, H. C., Cooper, M. J., Cogswell, P. C., Duncan, J. A., Ting, J. P., & Baldwin, A. S. (2008). Akt-dependent regulation of NF- $\kappa$ B is controlled by mTOR and Raptor in association with IKK. *Genes Dev.*, 22, 1490-1500. doi: 1410.1101/gad.1662308.

Darzynkiewicz, Z., Zhao, H., Halicka, H. D., Li, J., Lee, Y. S., Hsieh, T. C., & Wu, J. M. (2014). In search of antiaging modalities: Evaluation of mTOR- and ROS/DNA damage-signaling by cytometry. *Cytometry A*, 85, 386-399.

Davies, B. R., Greenwood, H., Dudley, P., Crafter, C., Yu, D. H., Zhang, J., Li, J., Gao, B., Ji, Q., Maynard, J., Ricketts, S. A., Cross, D., Cosulich, S., Chresta, C. C., Page, K., Yates, J., Lane, C., Watson, R., Luke, R., Ogilvie, D., & Pass, M. (2012). Preclinical pharmacology of AZD5363, an inhibitor of AKT: pharmacodynamics, antitumor activity, and correlation of monotherapy activity with genetic background. *Mol Cancer Ther*, 11, 873-887.

De, P., Dey, N., Terakedis, B., Bergsagel, P. L., Li, Z. H., Mahadevan, D., Garlich, J. R., Trudel, S., Makale, M. T., & Durden, D. L. (2013). An integrin-targeted, pan-isoform, phosphoinositide-3 kinase inhibitor, SF1126, has activity against multiple myeloma in vivo. *Cancer Chemother Pharmacol*, 71, 867-881.

Demetri, G. D., Chawla, S. P., Ray-Coquard, I., Le Cesne, A., Staddon, A. P., Milhem, M. M., Penel, N., Riedel, R. F., Bui-Nguyen, B., Cranmer, L. D., Reichardt, P., Bompas, E., Alcindor, T., Rushing, D., Song, Y., Lee, R. M., Ebbinghaus, S., Eid, J. E., Loewy, J. W., Haluska, F. G., Dodion, P. F., & Blay, J. Y. (2013). Results of an international randomized phase III trial of the mammalian target of rapamycin inhibitor ridaforolimus versus placebo to control metastatic sarcomas in patients after benefit from prior chemotherapy. *J Clin Oncol*, 31, 2485-2492.

Du, K., & Montminy, M. (1998). CREB is a regulatory target for the protein kinase Akt/PKB. *J Biol Chem.*, 273, 32377-32379.

Duenas, M., Martinez-Fernandez, M., Garcia-Escudero, R., Villacampa, F., Marques, M., Saiz-Ladera, C., Duarte, J., Martinez, V., Gomez, M. J., Martin, M. L., Fernandez, M., Castellano, D., Real, F. X., Rodriguez-Peralto, J. L., De La Rosa, F., & Paramio, J. M. (2013). PIK3CA gene alterations in bladder cancer are frequent and associate with reduced recurrence in non-muscle invasive tumors. *Mol Carcinog*, 2013, 22125.

Egan, D., Kim, J., Shaw, R. J., & Guan, K. L. (2011). The autophagy initiating kinase ULK1 is regulated via opposing phosphorylation by AMPK and mTOR. *Autophagy.*, 7, 643-644. Epub 2011 Jun 2011.

Engelman, J. A. (2009). Targeting PI3K signalling in cancer: opportunities, challenges and limitations. *Nat Rev Cancer.*, 9, 550-562. doi: 510.1038/nrc2664.

Engelman, J. A., Luo, J., & Cantley, L. C. (2006). The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet.*, 7, 606-619.

Fajkovic, H., Halpern, J. A., Cha, E. K., Bahadori, A., Chromecki, T. F., Karakiewicz, P. I., Breinl, E., Merseburger, A. S., & Shariat, S. F. (2011). Impact of gender on bladder cancer incidence, staging, and prognosis. *World J Urol*, 29, 457-463.

Falcon, B. L., Barr, S., Gokhale, P. C., Chou, J., Fogarty, J., Depeille, P., Miglarese, M., Epstein, D. M., & McDonald, D. M. (2011). Reduced VEGF production, angiogenesis, and vascular regrowth contribute to the antitumor properties of dual mTORC1/mTORC2 inhibitors. *Cancer Res*, 71, 1573-1583.

Fazio, N., Granberg, D., Grossman, A., Saletan, S., Klimovsky, J., Panneerselvam, A., & Wolin, E. M. (2013). Everolimus plus octreotide long-acting repeatable in patients with advanced lung neuroendocrine tumors: analysis of the phase 3, randomized, placebo-controlled RADIANT-2 study. *Chest.*, 143, 955-962.

Fechner, G., Classen, K., Schmidt, D., Hauser, S., & Muller, S. C. (2009). Rapamycin inhibits in vitro growth and release of angiogenic factors in human bladder cancer. *Urology.*, 73, 665-668; discussion 668-669. doi: 610.1016/j.urology.2008.1009.1070. Epub 2008 Dec 1011.

Feldman, R. I., Wu, J. M., Polokoff, M. A., Kochanny, M. J., Dinter, H., Zhu, D., Biroc, S. L., Alicke, B., Bryant, J., Yuan, S., Buckman, B. O., Lentz, D., Ferrer, M., Whitlow, M., Adler, M., Finster, S., Chang, Z., & Arnaiz, D. O. (2005). Novel small molecule inhibitors of 3-phosphoinositide-dependent kinase-1. *J Biol Chem.*, 280, 19867-19874. Epub 12005 Mar 19816.

Fokas, E., Yoshimura, M., Prevo, R., Higgins, G., Hackl, W., Maira, S. M., Bernhard, E. J., McKenna, W. G., & Muschel, R. J. (2012). NVP-BEZ235 and NVP-BGT226, dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitors, enhance tumor and endothelial cell radiosensitivity. *Radiat Oncol*, 7, 7-48.

Franz, D. N. (2013). Everolimus for astrocytomas in tuberous sclerosis - author's reply. *Lancet*, 381, 60837-60835.

Frias, M. A., Thoreen, C. C., Jaffe, J. D., Schroder, W., Sculley, T., Carr, S. A., & Sabatini, D. M. (2006). mSin1 is necessary for Akt/PKB phosphorylation, and its isoforms define three distinct mTORC2s. *Curr Biol.*, 16, 1865-1870. Epub 2006 Aug 1817.

Furet, P., Guagnano, V., Fairhurst, R. A., Imbach-Weese, P., Bruce, I., Knapp, M., Fritsch, C., Blasco, F., Blanz, J., Aichholz, R., Hamon, J., Fabbro, D., & Caravatti, G. (2013). Discovery of NVP-BYL719 a potent and selective phosphatidylinositol-3 kinase alpha inhibitor selected for clinical evaluation. *Bioorg Med Chem Lett*, 23, 3741-3748.

Furman, R. R., Sharman, J. P., Coutre, S. E., Cheson, B. D., Pagel, J. M., Hillmen, P., Barrientos, J. C., Zelenetz, A. D., Kipps, T. J., Flinn, I., Ghia, P., Eradat, H., Ervin, T., Lamanna, N., Coiffier, B., Pettitt, A. R., Ma, S., Stilgenbauer, S., Cramer, P., Aiello, M., Johnson, D. M., Miller, L. L., Li, D., Jahn, T. M., Dansey, R. D., Hallek, M., & O'Brien, S. M. (2014). Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. *N Engl J Med*, 370, 997-1007.

Gao, T., Furnari, F., & Newton, A. C. (2005). PHLPP: a phosphatase that directly dephosphorylates Akt, promotes apoptosis, and suppresses tumor growth. *Mol Cell.*, 18, 13-24.

Garcia-Martinez, J. M., & Alessi, D. R. (2008). mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum- and glucocorticoid-induced protein kinase 1 (SGK1). *Biochem J.*, 416, 375-385. doi: 310.1042/BJ20081668.

Gerullis, H., Eimer, C., Ecke, T. H., Georgas, E., Freitas, C., Kastenholz, S., Arndt, C., Heusch, C., & Otto, T. (2012). A phase II trial of temsirolimus in second-line metastatic urothelial cancer. *Med Oncol*, 29, 2870-2876.

Goebell, P. J., & Knowles, M. A. (2010). Bladder cancer or bladder cancers? Genetically distinct malignant conditions of the urothelium. *Urol Oncol*, 28, 409-428.

Groen, H. J., Sietsma, H., Vincent, A., Hochstenbag, M. M., van Putten, J. W., van den Berg, A., Dalesio, O., Biesma, B., Smit, H. J., Termeer, A., Hiltermann, T. J., van den Borne, B. E., & Schramel, F. M. (2011). Randomized, placebo-controlled phase III study of docetaxel plus carboplatin with celecoxib and cyclooxygenase-2 expression as a biomarker for patients with advanced non-small-cell lung cancer: the NVALT-4 study. *J Clin Oncol*, 29, 4320-4326.

Gui, Y., Guo, G., Huang, Y., Hu, X., Tang, A., Gao, S., Wu, R., Chen, C., Li, X., Zhou, L., He, M., Li, Z., Sun, X., Jia, W., Chen, J., Yang, S., Zhou, F., Zhao, X., Wan, S., Ye, R., Liang, C., Liu, Z., Huang, P., Liu, C., Jiang, H., Wang, Y., Zheng, H., Sun, L., Liu, X., Jiang, Z., Feng, D., Wu, S., Zou, J., Zhang, Z., Yang, R., Zhao, J., Xu, C., Yin, W., Guan, Z., Ye, J., Zhang, H., Li, J., Kristiansen, K., Nickerson, M. L., Theodorescu, D., Li, Y., Zhang, X., Li, S., Wang, J., Yang, H., & Cai, Z. (2011). Frequent mutations of chromatin remodeling genes in transitional cell carcinoma of the bladder. *Nat Genet*, 43, 875-878.

Guo, G., Sun, X., Chen, C., Wu, S., Huang, P., Li, Z., Dean, M., Huang, Y., Jia, W., Zhou, Q., Tang, A., Yang, Z., Li, X., Song, P., Zhao, X., Ye, R., Zhang, S., Lin, Z., Qi, M., Wan, S., Xie, L., Fan, F., Nickerson, M. L., Zou, X., Hu, X., Xing, L., Lv, Z., Mei, H., Gao, S., Liang, C., Gao, Z., Lu, J., Yu, Y., Liu, C., Li, L., Fang, X., Jiang, Z., Yang, J., Li, C., Chen, J., Zhang, F., Lai, Y., Zhou, F., Chen, H., Chan, H. C., Tsang, S., Theodorescu, D., Li, Y., Zhang, X., Wang, J., Yang, H., Gui, Y., & Cai, Z. (2013). Whole-genome and whole-exome sequencing of bladder cancer identifies frequent alterations in genes involved in sister chromatid cohesion and segregation. *Nat Genet*, 45, 1459-1463.

Guo, Y., Chekaluk, Y., Zhang, J., Du, J., Gray, N. S., Wu, C. L., & Kwiatkowski, D. J. (2013b). TSC1 involvement in bladder cancer: diverse effects and therapeutic implications. *J Pathol*, 230, 17-27.

Hara, K., Maruki, Y., Long, X., Yoshino, K., Oshiro, N., Hidayat, S., Tokunaga, C., Avruch, J., & Yonezawa, K. (2002). Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. *Cell*, 110, 177-189.

Harrington, L. S., Findlay, G. M., Gray, A., Tolkacheva, T., Wigfield, S., Rebholz, H., Barnett, J., Leslie, N. R., Cheng, S., Shepherd, P. R., Gout, I., Downes, C. P., & Lamb, R. F. (2004). The TSC1-2 tumor suppressor controls insulin-PI3K signaling via regulation of IRS proteins. *J Cell Biol.*, 166, 213-223. Epub 2004 Jul 2012.

Hay, N. (2005). The Akt-mTOR tango and its relevance to cancer. *Cancer Cell.*, 8, 179-183.

Heffron, T. P., Berry, M., Castanedo, G., Chang, C., Chuckowree, I., Dotson, J., Folkes, A., Gunzner, J., Lesnick, J. D., Lewis, C., Mathieu, S., Nonomiya, J., Olivero, A., Pang, J., Peterson, D., Salphati, L., Sampath, D., Sideris, S., Sutherlin, D. P., Tsui, V., Wan, N. C., Wang, S., Wong, S., & Zhu, B. Y. (2010). Identification of GNE-477, a potent and efficacious dual PI3K/mTOR inhibitor. *Bioorg Med Chem Lett*, 20, 2408-2411.

Heitman, J., Movva, N. R., & Hall, M. N. (1991). Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science.*, 253, 905-909.

Hess, G., Herbrecht, R., Romaguera, J., Verhoef, G., Crump, M., Gisselbrecht, C., Laurell, A., Offner, F., Strahs, A., Berkenblit, A., Hanushevsky, O., Clancy, J., Hewes, B., Moore, L., & Coiffier, B. (2009). Phase III study to evaluate temsirolimus compared with investigator's choice therapy for the treatment of relapsed or refractory mantle cell lymphoma. *J Clin Oncol.*, 27, 3822-3829. doi: 3810.1200/JCO.2008.3820.7977. Epub 2009 Jul 3826.

Hoang, B., Frost, P., Shi, Y., Belanger, E., Benavides, A., Pezeshkpour, G., Cappia, S., Guglielmelli, T., Gera, J., & Lichtenstein, A. (2010). Targeting TORC2 in multiple myeloma with a new mTOR kinase inhibitor. *Blood*, 116, 4560-4568.

Hornberger, T. A., Sukhija, K. B., Wang, X. R., & Chien, S. (2007). mTOR is the rapamycin-sensitive kinase that confers mechanically-induced phosphorylation of the hydrophobic motif site Thr(389) in p70(S6k). *FEBS Lett.*, 581, 4562-4566. Epub 2007 Aug 4531.

Huo, H. Z., Zhou, Z. Y., Wang, B., Qin, J., Liu, W. Y., & Gu, Y. (2014). Dramatic suppression of colorectal cancer cell growth by the dual mTORC1 and mTORC2 inhibitor AZD-2014. *Biochem Biophys Res Commun*, 443, 406-412.

Hutson, T. E., Escudier, B., Esteban, E., Bjarnason, G. A., Lim, H. Y., Pittman, K. B., Senico, P., Niethammer, A., Lu, D. R., Hariharan, S., & Motzer, R. J. (2014). Randomized phase III trial of temsirolimus versus sorafenib as second-line therapy after sunitinib in patients with metastatic renal cell carcinoma. *J Clin Oncol*, 32, 760-767.

Ihle, N. T., Williams, R., Chow, S., Chew, W., Berggren, M. I., Paine-Murrieta, G., Minion, D. J., Halter, R. J., Wipf, P., Abraham, R., Kirkpatrick, L., & Powis, G. (2004). Molecular pharmacology and antitumor activity of PX-866, a novel inhibitor of phosphoinositide-3-kinase signaling. *Mol Cancer Ther.*, 3, 763-772.

Inoki, K., Li, Y., Xu, T., & Guan, K. L. (2003). Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes Dev.*, 17, 1829-1834. Epub 2003 Jul 1817.

Inoki, K., Li, Y., Zhu, T., Wu, J., & Guan, K. L. (2002). TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nat Cell Biol.*, 4, 648-657.

Inoki, K., Zhu, T., & Guan, K. L. (2003). TSC2 mediates cellular energy response to control cell growth and survival. *Cell.*, 115, 577-590.

Iyer, G., Hanrahan, A. J., Milowsky, M. I., Al-Ahmadie, H., Scott, S. N., Janakiraman, M., Pirun, M., Sander, C., Socci, N. D., Ostrovnya, I., Viale, A., Heguy, A., Peng, L., Chan, T. A., Bochner, B., Bajorin, D. F., Berger, M. F., Taylor, B. S., & Solit, D. B. (2012). Genome sequencing identifies a basis for everolimus sensitivity. *Science*, 338, 221.

Iyer, G., Al-Ahmadie, H., Schultz, N., Hanrahan, A. J., Ostrovnya, I., Balar, A. V., Kim, P. H., Lin, O., Weinhold, N., Sander, C., Zabor, E. C., Janakiraman, M., Garcia-Grossman, I. R., Heguy, A., Viale, A., Bochner, B. H., Reuter, V. E., Bajorin, D. F., Milowsky, M. I., Taylor, B. S., & Solit, D. B. (2013).



Prevalence and co-occurrence of actionable genomic alterations in high-grade bladder cancer. *J Clin Oncol*, 31, 3133-3140.

Jacinto, E., Loewith, R., Schmidt, A., Lin, S., Ruegg, M. A., Hall, A., & Hall, M. N. (2004). Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat Cell Biol.*, 6, 1122-1128. Epub 2004 Oct 1123.

James, N. D., Sydes, M. R., Mason, M. D., Clarke, N. W., Anderson, J., Dearnaley, D. P., Dwyer, J., Jovic, G., Ritchie, A. W., Russell, J. M., Sanders, K., Thalmann, G. N., Bertelli, G., Birtle, A. J., O'Sullivan, J. M., Protheroe, A., Sheehan, D., Srihari, N., & Parmar, M. K. (2012). Celecoxib plus hormone therapy versus hormone therapy alone for hormone-sensitive prostate cancer: first results from the STAMPEDE multiarm, multistage, randomised controlled trial. *Lancet Oncol*, 13, 549-558.

Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E., & Forman, D. (2011). Global cancer statistics. *CA Cancer J Clin*, 61, 69-90. doi: 10.3322/caac.20107. Epub 22011 Feb 20104.

Kagan, J., Liu, J., Stein, J. D., Wagner, S. S., Babkowski, R., Grossman, B. H., & Katz, R. L. (1998). Cluster of allele losses within a 2.5 cM region of chromosome 10 in high-grade invasive bladder cancer. *Oncogene.*, 16, 909-913.

Kantor, A. F., Hartge, P., Hoover, R. N., & Fraumeni, J. F., Jr. (1988). Epidemiological characteristics of squamous cell carcinoma and adenocarcinoma of the bladder. *Cancer Res.*, 48, 3853-3855.

Katso, R., Okkenhaug, K., Ahmadi, K., White, S., Timms, J., & Waterfield, M. D. (2001). Cellular function of phosphoinositide 3-kinases: implications for development, homeostasis, and cancer. *Annu Rev Cell Dev Biol*, 17, 615-675.

Kim, D. H., Sarbassov, D. D., Ali, S. M., King, J. E., Latek, R. R., Erdjument-Bromage, H., Tempst, P., & Sabatini, D. M. (2002). mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell.*, 110, 163-175.

Kim, D. H., Sarbassov, D. D., Ali, S. M., Latek, R. R., Guntur, K. V., Erdjument-Bromage, H., Tempst, P., & Sabatini, D. M. (2003). GbetaL, a positive regulator of the rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR. *Mol Cell.*, 11, 895-904.

- Kim, J., Kundu, M., Viollet, B., & Guan, K. L. (2011). AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol*, 13, 132-141.
- Kim, J. J. (2012). Recent advances in treatment of advanced urothelial carcinoma. *Curr Urol Rep*, 13, 147-152.
- Knight, Z. A., Gonzalez, B., Feldman, M. E., Zunder, E. R., Goldenberg, D. D., Williams, O., Loewith, R., Stokoe, D., Balla, A., Toth, B., Balla, T., Weiss, W. A., Williams, R. L., & Shokat, K. M. (2006). A pharmacological map of the PI3-K family defines a role for p110alpha in insulin signaling. *Cell*, 125, 733-747. Epub 2006 Apr 2027.
- Knowles, M. A., Habuchi, T., Kennedy, W., & Cuthbert-Heavens, D. (2003). Mutation spectrum of the 9q34 tuberous sclerosis gene TSC1 in transitional cell carcinoma of the bladder. *Cancer Res.*, 63, 7652-7656.
- Knowles, M. A., Platt, F. M., Ross, R. L., & Hurst, C. D. (2009). Phosphatidylinositol 3-kinase (PI3K) pathway activation in bladder cancer. *Cancer Metastasis Rev.*, 28, 305-316. doi: 310.1007/s10555-10009-19198-10553.
- Korkolopoulou, P., Levidou, G., Trigka, E. A., Prekete, N., Karlou, M., Thymara, I., Sakellariou, S., Fragkou, P., Isaiadis, D., Pavlopoulos, P., Patsouris, E., & Saetta, A. A. (2012). A comprehensive immunohistochemical and molecular approach to the PI3K/AKT/mTOR (phosphoinositide 3-kinase/v-akt murine thymoma viral oncogene/mammalian target of rapamycin) pathway in bladder urothelial carcinoma. *BJU Int*, 110, 29.
- Krawczyk, J., Keane, N., Swords, R., O'Dwyer, M., Freeman, C. L., & Giles, F. J. (2013). Perifosine--a new option in treatment of acute myeloid leukemia? *Expert Opin Investig Drugs*, 22, 1315-1327.
- Lamming, D. W., Ye, L., Sabatini, D. M., & Baur, J. A. (2013). Rapalogs and mTOR inhibitors as anti-aging therapeutics. *J Clin Invest*, 123, 980-989.
- Lannutti, B. J., Meadows, S. A., Herman, S. E., Kashishian, A., Steiner, B., Johnson, A. J., Byrd, J. C., Tyner, J. W., Loriaux, M. M., Deininger, M., Druker, B. J., Puri, K. D., Ulrich, R. G., & Giese, N. A. (2011). CAL-101, a p110delta selective phosphatidylinositol-3-kinase inhibitor for the treatment of B-cell malignancies, inhibits PI3K signaling and cellular viability. *Blood*, 117, 591-594.

Liang, J., Zubovitz, J., Petrocelli, T., Kotchetkov, R., Connor, M. K., Han, K., Lee, J. H., Ciarallo, S., Catzavelos, C., Beniston, R., Franssen, E., & Slingerland, J. M. (2002). PKB/Akt phosphorylates p27, impairs nuclear import of p27 and opposes p27-mediated G1 arrest. *Nat Med.*, 8, 1153-1160. Epub 2002 Sep 1116.

Lin, C. C., Hsu, C. H., Pu, Y. S., & Vogelzang, N. J. (2008). Systemic therapy for metastatic urothelial carcinoma. *BJU Int.*, 101, 795-803. Epub 2007 Dec 2005.

Lin, J., Sampath, D., Nannini, M. A., Lee, B. B., Degtyarev, M., Oeh, J., Savage, H., Guan, Z., Hong, R., Kassees, R., Lee, L. B., Risom, T., Gross, S., Liederer, B. M., Koeppen, H., Skelton, N. J., Wallin, J. J., Belvin, M., Punnoose, E., Friedman, L. S., & Lin, K. (2013). Targeting activated Akt with GDC-0068, a novel selective Akt inhibitor that is efficacious in multiple tumor models. *Clin Cancer Res*, 19, 1760-1772.

Lindgren, D., Sjudahl, G., Lauss, M., Staaf, J., Chebil, G., Lovgren, K., Gudjonsson, S., Liedberg, F., Patschan, O., Mansson, W., Ferno, M., & Hoglund, M. (2012). Integrated genomic and gene expression profiling identifies two major genomic circuits in urothelial carcinoma. *PLoS One*, 7, e38863.

Liu, N., Rowley, B. R., Bull, C. O., Schneider, C., Haegebarth, A., Schatz, C. A., Fracasso, P. R., Wilkie, D. P., Hentemann, M., Wilhelm, S. M., Scott, W. J., Mumberg, D., & Ziegelbauer, K. (2013). BAY 80-6946 is a highly selective intravenous PI3K inhibitor with potent p110alpha and p110delta activities in tumor cell lines and xenograft models. *Mol Cancer Ther*, 12, 2319-2330.

Liu, P., Cheng, H., Roberts, T. M., & Zhao, J. J. (2009). Targeting the phosphoinositide 3-kinase pathway in cancer. *Nat Rev Drug Discov.*, 8, 627-644. doi: 610.1038/nrd2926.

Liu, Q., Wang, J., Kang, S. A., Thoreen, C. C., Hur, W., Ahmed, T., Sabatini, D. M., & Gray, N. S. (2011). Discovery of 9-(6-aminopyridin-3-yl)-1-(3-(trifluoromethyl)phenyl)benzo[h][1,6]naphthyridin-2(1H)-one (Torin2) as a potent, selective, and orally available mammalian target of rapamycin (mTOR) inhibitor for treatment of cancer. *J Med Chem*, 54, 1473-1480.

Liu, Q., Xu, C., Kirubakaran, S., Zhang, X., Hur, W., Liu, Y., Kwiatkowski, N. P., Wang, J., Westover, K. D., Gao, P., Ercan, D., Niepel, M., Thoreen, C. C., Kang, S. A., Patricelli, M. P., Wang, Y., Tupper,

T., Altabef, A., Kawamura, H., Held, K. D., Chou, D. M., Elledge, S. J., Janne, P. A., Wong, K. K., Sabatini, D. M., & Gray, N. S. (2013). Characterization of Torin2, an ATP-competitive inhibitor of mTOR, ATM, and ATR. *Cancer Res*, 73, 2574-2586.

Liu, X., Shi, Y., Woods, K. W., Hessler, P., Kroeger, P., Wilsbacher, J., Wang, J., Wang, J. Y., Li, C., Li, Q., Rosenberg, S. H., Giranda, V. L., & Luo, Y. (2008). Akt inhibitor a-443654 interferes with mitotic progression by regulating aurora a kinase expression. *Neoplasia*, 10, 828-837.

Lopez-Knowles, E., Hernandez, S., Malats, N., Kogevinas, M., Lloreta, J., Carrato, A., Tardon, A., Serra, C., & Real, F. X. (2006). PIK3CA mutations are an early genetic alteration associated with FGFR3 mutations in superficial papillary bladder tumors. *Cancer Res.*, 66, 7401-7404.

Luo, Y., Shoemaker, A. R., Liu, X., Woods, K. W., Thomas, S. A., de Jong, R., Han, E. K., Li, T., Stoll, V. S., Powlas, J. A., Oleksijew, A., Mitten, M. J., Shi, Y., Guan, R., McGonigal, T. P., Klinghofer, V., Johnson, E. F., Levenson, J. D., Bouska, J. J., Mamo, M., Smith, R. A., Gramling-Evans, E. E., Zinker, B. A., Mika, A. K., Nguyen, P. T., Oltersdorf, T., Rosenberg, S. H., Li, Q., & Giranda, V. L. (2005). Potent and selective inhibitors of Akt kinases slow the progress of tumors in vivo. *Mol Cancer Ther.*, 4, 977-986.

Ma, L., Chen, Z., Erdjument-Bromage, H., Tempst, P., & Pandolfi, P. P. (2005). Phosphorylation and functional inactivation of TSC2 by Erk implications for tuberous sclerosis and cancer pathogenesis. *Cell.*, 121, 179-193.

Mahalingam, D., Medina, E. C., Esquivel, J. A., 2nd, Espitia, C. M., Smith, S., Oberheu, K., Swords, R., Kelly, K. R., Mita, M. M., Mita, A. C., Carew, J. S., Giles, F. J., & Nawrocki, S. T. (2010). Vorinostat enhances the activity of temsirolimus in renal cell carcinoma through suppression of survivin levels. *Clin Cancer Res*, 16, 141-153.

Maira, S. M., Pecchi, S., Huang, A., Burger, M., Knapp, M., Sterker, D., Schnell, C., Guthy, D., Nagel, T., Wiesmann, M., Brachmann, S., Fritsch, C., Dorsch, M., Chene, P., Shoemaker, K., De Pover, A., Menezes, D., Martiny-Baron, G., Fabbro, D., Wilson, C. J., Schlegel, R., Hofmann, F., Garcia-Echeverria, C., Sellers, W. R., & Voliva, C. F. (2012). Identification and characterization of NVP-BKM120, an orally available pan-class I PI3-kinase inhibitor. *Mol Cancer Ther*, 11, 317-328.

Mallon, R., Hollander, I., Feldberg, L., Lucas, J., Soloveva, V., Venkatesan, A., Dehnhardt, C., Delos Santos, E., Chen, Z., Dos Santos, O., Ayril-Kaloustian, S., & Gibbons, J. (2010). Antitumor efficacy profile of PKI-402, a dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor. *Mol Cancer Ther*, 9, 976-984.

Manara, M. C., Nicoletti, G., Zambelli, D., Ventura, S., Guerzoni, C., Landuzzi, L., Lollini, P. L., Maira, S. M., Garcia-Echeverria, C., Mercuri, M., Picci, P., & Scotlandi, K. (2010). NVP-BEZ235 as a new therapeutic option for sarcomas. *Clin Cancer Res*, 16, 530-540.

Manning, B. D., & Cantley, L. C. (2003). Rheb fills a GAP between TSC and TOR. *Trends Biochem Sci.*, 28, 573-576.

Mansure, J. J., Nassim, R., Chevalier, S., Rocha, J., Scarlata, E., & Kassouf, W. (2009). Inhibition of mammalian target of rapamycin as a therapeutic strategy in the management of bladder cancer. *Cancer Biol Ther.*, 8, 2339-2347. Epub 2009 Dec 2332.

Marcus, P. M., Hayes, R. B., Vineis, P., Garcia-Closas, M., Caporaso, N. E., Autrup, H., Branch, R. A., Brockmoller, J., Ishizaki, T., Karakaya, A. E., Ladero, J. M., Mommsen, S., Okkels, H., Romkes, M., Roots, I., & Rothman, N. (2000). Cigarette smoking, N-acetyltransferase 2 acetylation status, and bladder cancer risk: a case-series meta-analysis of a gene-environment interaction. *Cancer Epidemiol Biomarkers Prev.*, 9, 461-467.

Marcus, P. M., Vineis, P., & Rothman, N. (2000). NAT2 slow acetylation and bladder cancer risk: a meta-analysis of 22 case-control studies conducted in the general population. *Pharmacogenetics.*, 10, 115-122.

Markman, B., Dienstmann, R., & Tabernero, J. (2010). Targeting the PI3K/Akt/mTOR pathway--beyond rapalogs. *Oncotarget.*, 1, 530-543.

Markman, B., Tabernero, J., Krop, I., Shapiro, G. I., Siu, L., Chen, L. C., Mita, M., Melendez Cuero, M., Stutvoet, S., Birle, D., Anak, O., Hackl, W., & Baselga, J. (2012). Phase I safety, pharmacokinetic, and pharmacodynamic study of the oral phosphatidylinositol-3-kinase and mTOR inhibitor BGT226 in patients with advanced solid tumors. *Ann Oncol*, 23, 2399-2408.

Martin, J., Masri, J., Bernath, A., Nishimura, R. N., & Gera, J. (2008). Hsp70 associates with Rictor and is required for mTORC2 formation and activity. *Biochem Biophys Res Commun.*, 372, 578-583. doi: 510.1016/j.bbrc.2008.1005.1086. Epub 2008 May 1027.

Maruyama, R., Toyooka, S., Toyooka, K. O., Harada, K., Virmani, A. K., Zochbauer-Muller, S., Farinas, A. J., Vakar-Lopez, F., Minna, J. D., Sagalowsky, A., Czerniak, B., & Gazdar, A. F. (2001). Aberrant promoter methylation profile of bladder cancer and its relationship to clinicopathological features. *Cancer Res*, 61, 8659-8663.

Matic, M., Pekmezovic, T., Djukic, T., Mimic-Oka, J., Dragicevic, D., Krivic, B., Suvakov, S., Savic-Radojevic, A., Pljesa-Ercegovac, M., Tulic, C., Coric, V., & Simic, T. (2013). GSTA1, GSTM1, GSTP1, and GSTT1 polymorphisms and susceptibility to smoking-related bladder cancer: a case-control study. *Urol Oncol*, 31, 1184-1192.

McCubrey, J. A., Steelman, L. S., Chappell, W. H., Abrams, S. L., Franklin, R. A., Montalto, G., Cervello, M., Libra, M., Candido, S., Malaponte, G., Mazzarino, M. C., Fagone, P., Nicoletti, F., Basecke, J., Mijatovic, S., Maksimovic-Ivanic, D., Milella, M., Tafuri, A., Chiarini, F., Evangelisti, C., Cocco, L., & Martelli, A. M. (2012). Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR cascade inhibitors: how mutations can result in therapy resistance and how to overcome resistance. *Oncotarget*, 3, 1068-1111.

McCubrey, J. A., Steelman, L. S., Chappell, W. H., Abrams, S. L., Montalto, G., Cervello, M., Nicoletti, F., Fagone, P., Malaponte, G., Mazzarino, M. C., Candido, S., Libra, M., Basecke, J., Mijatovic, S., Maksimovic-Ivanic, D., Milella, M., Tafuri, A., Cocco, L., Evangelisti, C., Chiarini, F., & Martelli, A. M. (2012). Mutations and deregulation of Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR cascades which alter therapy response. *Oncotarget.*, 3, 954-987.

Mendoza, M. C., Er, E. E., & Blenis, J. (2011). The Ras-ERK and PI3K-mTOR pathways: cross-talk and compensation. *Trends Biochem Sci*, 36, 320-328.

Mhaweche-Fauceglia, P., Cheney, R. T., & Schwaller, J. (2006). Genetic alterations in urothelial bladder carcinoma: an updated review. *Cancer*, 106, 1205-1216.

Milowsky, M. I., Iyer, G., Regazzi, A. M., Al-Ahmadie, H., Gerst, S. R., Ostrovnaya, I., Gellert, L. L., Kaplan, R., Garcia-Grossman, I. R., Pendse, D., Balar, A. V., Flaherty, A. M., Trout, A., Solit, D. B., & Bajorin, D. F. (2013). Phase II study of everolimus in metastatic urothelial cancer. *BJU Int*, 112, 462-470.

Mirzoeva, O. K., Hann, B., Hom, Y. K., Debnath, J., Aftab, D., Shokat, K., & Korn, W. M. (2011). Autophagy suppression promotes apoptotic cell death in response to inhibition of the PI3K-mTOR pathway in pancreatic adenocarcinoma. *J Mol Med*, 89, 877-889.

Motzer, R. J., Escudier, B., Oudard, S., Hutson, T. E., Porta, C., Bracarda, S., Grunwald, V., Thompson, J. A., Figlin, R. A., Hollaender, N., Kay, A., & Ravaud, A. (2010). Phase 3 trial of everolimus for metastatic renal cell carcinoma : final results and analysis of prognostic factors. *Cancer*, 116, 4256-4265.

Munster, P., van der Noll, R., Voest, E., Specht, J., Werner, T. L., Dees, E. C., Tan, A. R., Daud, A., Schellens, J., Lolkema, M. P., Griffin, M., Agarwal, N., Falchook, G. S., Kleha, J. F., Durante, M., Smith, D. A., Adams, L., Greshock, J., Morris, S. R., & Kurzrock, R. (2012). PI3K kinase inhibitor GSK2126458 (GSK458): clinical activity in select patient (pt) populations defined by predictive markers (study P3K112826). *Ann Oncol*, 23, abstract 4420.

Najafov, A., Sommer, E. M., Axten, J. M., Deyoung, M. P., & Alessi, D. R. (2011). Characterization of GSK2334470, a novel and highly specific inhibitor of PDK1. *Biochem J*, 433, 357-369.

Nazio, F., Strappazzon, F., Antonioli, M., Bielli, P., Cianfanelli, V., Bordi, M., Gretzmeier, C., Dengjel, J., Piacentini, M., Fimia, G. M., & Cecconi, F. (2013). mTOR inhibits autophagy by controlling ULK1 ubiquitylation, self-association and function through AMBRA1 and TRAF6. *Nat Cell Biol*, 15, 406-416.

Neshat, M. S., Mellingshoff, I. K., Tran, C., Stiles, B., Thomas, G., Petersen, R., Frost, P., Gibbons, J. J., Wu, H., & Sawyers, C. L. (2001). Enhanced sensitivity of PTEN-deficient tumors to inhibition of FRAP/mTOR. *Proc Natl Acad Sci U S A.*, 98, 10314-10319. Epub 12001 Aug 10314.

Network, T. C. G. A. R. (2014). Comprehensive molecular characterization of urothelial bladder carcinoma. *Nature*, 2014.

Oh, W. J., & Jacinto, E. (2011). mTOR complex 2 signaling and functions. *Cell Cycle*, 10, 2305-2316. Epub 2011 Jul 2315.

Oh, W. J., Wu, C. C., Kim, S. J., Facchinetti, V., Julien, L. A., Finlan, M., Roux, P. P., Su, B., & Jacinto, E. (2010). mTORC2 can associate with ribosomes to promote cotranslational phosphorylation and stability of nascent Akt polypeptide. *Embo J*, 29, 3939-3951.

Ohtsu, A., Ajani, J. A., Bai, Y. X., Bang, Y. J., Chung, H. C., Pan, H. M., Sahmoud, T., Shen, L., Yeh, K. H., Chin, K., Muro, K., Kim, Y. H., Ferry, D., Tebbutt, N. C., Al-Batran, S. E., Smith, H., Costantini, C., Rizvi, S., Lebwohl, D., & Van Cutsem, E. (2013). Everolimus for previously treated advanced gastric cancer: results of the randomized, double-blind, phase III GRANITE-1 study. *J Clin Oncol*, 31, 3935-3943.

Okajima, E., Hiramatsu, T., Iriya, K., Ijuin, M., & Matsushima, S. (1975). Effects of sex hormones on development of urinary bladder tumours in rats induced by N-butyl-N-(4-hydroxybutyl) nitrosamine. *Urol Res.*, 3, 73-79.

Ozes, O. N., Mayo, L. D., Gustin, J. A., Pfeffer, S. R., Pfeffer, L. M., & Donner, D. B. (1999). NF-kappaB activation by tumour necrosis factor requires the Akt serine-threonine kinase. *Nature.*, 401, 82-85.

Pause, A., Belsham, G. J., Gingras, A. C., Donze, O., Lin, T. A., Lawrence, J. C., Jr., & Sonenberg, N. (1994). Insulin-dependent stimulation of protein synthesis by phosphorylation of a regulator of 5'-cap function. *Nature.*, 371, 762-767.

Pavel, M. E., Hainsworth, J. D., Baudin, E., Peeters, M., Horsch, D., Winkler, R. E., Klimovsky, J., Lebwohl, D., Jehl, V., Wolin, E. M., Oberg, K., Van Cutsem, E., & Yao, J. C. (2011). Everolimus plus octreotide long-acting repeatable for the treatment of advanced neuroendocrine tumours associated with carcinoid syndrome (RADIANT-2): a randomised, placebo-controlled, phase 3 study. *Lancet*, 378, 2005-2012.

Peterson, T. R., Laplante, M., Thoreen, C. C., Sancak, Y., Kang, S. A., Kuehl, W. M., Gray, N. S., & Sabatini, D. M. (2009). DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma



cells and required for their survival. *Cell.*, 137, 873-886. doi: 810.1016/j.cell.2009.1003.1046. Epub 2009 May 1014.

Pike, K. G., Malagu, K., Hummersone, M. G., Menear, K. A., Duggan, H. M., Gomez, S., Martin, N. M., Ruston, L., Pass, S. L., & Pass, M. (2013). Optimization of potent and selective dual mTORC1 and mTORC2 inhibitors: the discovery of AZD8055 and AZD2014. *Bioorg Med Chem Lett*, 23, 1212-1216.

Platt, F. M., Hurst, C. D., Taylor, C. F., Gregory, W. M., Harnden, P., & Knowles, M. A. (2009). Spectrum of phosphatidylinositol 3-kinase pathway gene alterations in bladder cancer. *Clin Cancer Res.*, 15, 6008-6017. doi: 6010.1158/1078-0432.CCR-6009-0898. Epub 2009 Sep 6029.

Ploeg, M., Aben, K. K., & Kiemeny, L. A. (2009). The present and future burden of urinary bladder cancer in the world. *World J Urol.*, 27, 289-293. doi: 210.1007/s00345-00009-00383-00343. Epub 02009 Feb 00315.

Podsypanina, K., Lee, R. T., Politis, C., Hennessy, I., Crane, A., Puc, J., Neshat, M., Wang, H., Yang, L., Gibbons, J., Frost, P., Dreisbach, V., Blenis, J., Gaciong, Z., Fisher, P., Sawyers, C., Hedrick-Ellenson, L., & Parsons, R. (2001). An inhibitor of mTOR reduces neoplasia and normalizes p70/S6 kinase activity in *Pten*<sup>+/-</sup> mice. *Proc Natl Acad Sci U S A.*, 98, 10320-10325. Epub 12001 Aug 10314.

Ponzo, M. G., & Park, M. (2010). The Met receptor tyrosine kinase and basal breast cancer. *Cell Cycle*, 9, 1043-1050.

Populo, H., Lopes, J. M., & Soares, P. (2012). The mTOR Signalling Pathway in Human Cancer. *Int J Mol Sci*, 13, 1886-1918.

Prasad, G., Sottero, T., Yang, X., Mueller, S., James, C. D., Weiss, W. A., Polley, M. Y., Ozawa, T., Berger, M. S., Aftab, D. T., Prados, M. D., & Haas-Kogan, D. A. (2011). Inhibition of PI3K/mTOR pathways in glioblastoma and implications for combination therapy with temozolomide. *Neuro Oncol*, 13, 384-392.

Prat, E., Bernues, M., Caballin, M. R., Egozcue, J., Gelabert, A., & Miro, R. (2001). Detection of chromosomal imbalances in papillary bladder tumors by comparative genomic hybridization. *Urology*, 57, 986-992.

Prevo, R., Deutsch, E., Sampson, O., Diplexcito, J., Cengel, K., Harper, J., O'Neill, P., McKenna, W. G., Patel, S., & Bernhard, E. J. (2008). Class I PI3 kinase inhibition by the pyridinylfuranopyrimidine inhibitor PI-103 enhances tumor radiosensitivity. *Cancer Res.*, 68, 5915-5923. doi: 5910.1158/0008-5472.CAN-5908-0757.

Pymar, L. S., Platt, F. M., Askham, J. M., Morrison, E. E., & Knowles, M. A. (2008). Bladder tumour-derived somatic TSC1 missense mutations cause loss of function via distinct mechanisms. *Hum Mol Genet.*, 17, 2006-2017. doi: 2010.1093/hmg/ddn2098. Epub 2008 Apr 2007.

Raynaud, F. I., Eccles, S., Clarke, P. A., Hayes, A., Nutley, B., Alix, S., Henley, A., Di-Stefano, F., Ahmad, Z., Guillard, S., Bjerke, L. M., Kelland, L., Valenti, M., Patterson, L., Gowan, S., de Haven Brandon, A., Hayakawa, M., Kaizawa, H., Koizumi, T., Ohishi, T., Patel, S., Saghir, N., Parker, P., Waterfield, M., & Workman, P. (2007). Pharmacologic characterization of a potent inhibitor of class I phosphatidylinositol 3-kinases. *Cancer Res.*, 67, 5840-5850.

Rhodes, N., Heerding, D. A., Duckett, D. R., Eberwein, D. J., Knick, V. B., Lansing, T. J., McConnell, R. T., Gilmer, T. M., Zhang, S. Y., Robell, K., Kahana, J. A., Geske, R. S., Kleymenova, E. V., Choudhry, A. E., Lai, Z., Leber, J. D., Minthorn, E. A., Strum, S. L., Wood, E. R., Huang, P. S., Copeland, R. A., & Kumar, R. (2008). Characterization of an Akt kinase inhibitor with potent pharmacodynamic and antitumor activity. *Cancer Res.*, 68, 2366-2374. doi: 2310.1158/0008-5472.CAN-2307-5783.

Richter, J., Wagner, U., Schraml, P., Maurer, R., Alund, G., Knonagel, H., Moch, H., Mihatsch, M. J., Gasser, T. C., & Sauter, G. (1999). Chromosomal imbalances are associated with a high risk of progression in early invasive (pT1) urinary bladder cancer. *Cancer Res*, 59, 5687-5691.

Rini, B. I., Bellmunt, J., Clancy, J., Wang, K., Niethammer, A. G., Hariharan, S., & Escudier, B. (2014). Randomized phase III trial of temsirolimus and bevacizumab versus interferon alfa and bevacizumab in metastatic renal cell carcinoma: INTORACT trial. *J Clin Oncol*, 32, 752-759.

Rodon, J., Brana, I., Siu, L. L., De Jonge, M. J., Homji, N., Mills, D., Di Tomaso, E., Sarr, C., Trandafir, L., Massacesi, C., Eskens, F., & Bendell, J. C. (2014). Phase I dose-escalation and -expansion study of buparlisib (BKM120), an oral pan-Class I PI3K inhibitor, in patients with advanced solid tumors. *Invest New Drugs*, 2014, 21.

Rodon, J., Dienstmann, R., Serra, V., & Tabernero, J. (2013). Development of PI3K inhibitors: lessons learned from early clinical trials. *Nat Rev Clin Oncol*, 10, 143-153.

Ross, R. L., Askham, J. M., & Knowles, M. A. (2013). PIK3CA mutation spectrum in urothelial carcinoma reflects cell context-dependent signaling and phenotypic outputs. *Oncogene*, 32, 768-776.

Ross, R. L., Burns, J. E., Taylor, C. F., Mellor, P., Anderson, D. H., & Knowles, M. A. (2013b). Identification of Mutations in Distinct Regions of p85 Alpha in Urothelial Cancer. *PLoS One*, 8, 2013.

Sancak, Y., Thoreen, C. C., Peterson, T. R., Lindquist, R. A., Kang, S. A., Spooner, E., Carr, S. A., & Sabatini, D. M. (2007). PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. *Mol Cell.*, 25, 903-915.

Sarbassov, D. D., Ali, S. M., Kim, D. H., Guertin, D. A., Latek, R. R., Erdjument-Bromage, H., Tempst, P., & Sabatini, D. M. (2004). Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr Biol.*, 14, 1296-1302.

Sarbassov, D. D., Guertin, D. A., Ali, S. M., & Sabatini, D. M. (2005). Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science.*, 307, 1098-1101.

Sato, T., Nakashima, A., Guo, L., & Tamanoi, F. (2009). Specific activation of mTORC1 by Rheb G-protein in vitro involves enhanced recruitment of its substrate protein. *J Biol Chem.*, 284, 12783-12791. doi: 12710.11074/jbc.M809207200. Epub 809202009 Mar 809207219.

Seront, E., Pinto, A., Bouzin, C., Bertrand, L., Machiels, J. P., & Feron, O. (2013). PTEN deficiency is associated with reduced sensitivity to mTOR inhibitor in human bladder cancer through the unhampered feedback loop driving PI3K/Akt activation. *Br J Cancer*, 109, 1586-1592.

Seront, E., Rottey, S., Sautois, B., Kerger, J., D'Hondt, L. A., Verschaeve, V., Canon, J. L., Dopchie, C., Vandenbulcke, J. M., Whenham, N., Goeminne, J. C., Clausse, M., Verhoeven, D., Glorieux, P., Branders, S., Dupont, P., Schoonjans, J., Feron, O., & Machiels, J. P. (2012). Phase II study of everolimus in patients with locally advanced or metastatic transitional cell carcinoma of the urothelial tract: clinical activity, molecular response, and biomarkers. *Ann Oncol*, 23, 2663-2670. Epub 2012 Apr 2663.

Serra, V., Markman, B., Scaltriti, M., Eichhorn, P. J., Valero, V., Guzman, M., Botero, M. L., Llonch, E., Atzori, F., Di Cosimo, S., Maira, M., Garcia-Echeverria, C., Parra, J. L., Arribas, J., & Baselga, J. (2008). NVP-BEZ235, a dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits the growth of cancer cells with activating PI3K mutations. *Cancer Res.*, 68, 8022-8030. doi: 8010.1158/0008-5472.CAN-8008-1385.

Sgnaolin, V., Pereira, T. C., Bogo, M. R., Zanin, R., Battastini, A. M., Morrone, F. B., & Campos, M. M. (2013). Functional and molecular characterization of kinin B1 and B2 receptors in human bladder cancer: implication of the PI3Kgamma pathway. *Invest New Drugs*, 31, 812-822.

Shapiro, G. I., Rodon, J., Bedell, C., Kwak, E. L., Baselga, J., Brana, I., Pandya, S. S., Scheffold, C., Laird, A. D., Nguyen, L. T., Xu, Y., Egile, C., & Edelman, G. (2014). Phase I safety, pharmacokinetic, and pharmacodynamic study of SAR245408 (XL147), an oral pan-class I PI3K inhibitor, in patients with advanced solid tumors. *Clin Cancer Res*, 20, 233-245.

Shaw, R. J., & Cantley, L. C. (2006). Ras, PI(3)K and mTOR signalling controls tumour cell growth. *Nature.*, 441, 424-430.

Shi, Y., Gera, J., Hu, L., Hsu, J. H., Bookstein, R., Li, W., & Lichtenstein, A. (2002). Enhanced sensitivity of multiple myeloma cells containing PTEN mutations to CCI-779. *Cancer Res.*, 62, 5027-5034.

Shibata, T., Ohta, T., Tong, K. I., Kokubu, A., Odogawa, R., Tsuta, K., Asamura, H., Yamamoto, M., & Hirohashi, S. (2008). Cancer related mutations in NRF2 impair its recognition by Keap1-Cul3 E3 ligase and promote malignancy. *Proc Natl Acad Sci U S A.*, 105, 13568-13573. doi: 13510.11073/pnas.0806268105. Epub 0806262008 Aug 0806268129.

Shugg, R. P., Thomson, A., Tanabe, N., Kashishian, A., Steiner, B. H., Puri, K. D., Pereverzev, A., Lannutti, B. J., Jirik, F. R., Dixon, S. J., & Sims, S. M. (2013). Effects of isoform-selective phosphatidylinositol 3-kinase inhibitors on osteoclasts: actions on cytoskeletal organization, survival, and resorption. *J Biol Chem*, 288, 35346-35357.

Simon, R., Burger, H., Semjonow, A., Hertle, L., Terpe, H. J., & Bocker, W. (2000). Patterns of chromosomal imbalances in muscle invasive bladder cancer. *Int J Oncol.*, 17, 1025-1029.

Sio, T. T., Ko, J., Gudena, V. K., Verma, N., & Chaudhary, U. B. (2014). Chemotherapeutic and targeted biological agents for metastatic bladder cancer: A comprehensive review. *Int J Urol*, 2014, 12407.

Sjodahl, G., Lauss, M., Gudjonsson, S., Liedberg, F., Hallden, C., Chebil, G., Mansson, W., Hoglund, M., & Lindgren, D. (2011). A systematic study of gene mutations in urothelial carcinoma; inactivating mutations in TSC2 and PIK3R1. *PLoS One*, 6, 0018583.

Skopelitou, A. S., Gloustanou, G., Bai, M., & Huebner, K. (2001). FHIT gene expression in human urinary bladder transitional cell carcinomas. *In Vivo*, 15, 169-173.

Smith, J. A., Jr., Labasky, R. F., Cockett, A. T., Fracchia, J. A., Montie, J. E., & Rowland, R. G. (1999). Bladder cancer clinical guidelines panel summary report on the management of nonmuscle invasive bladder cancer (stages Ta, T1 and TIS). The American Urological Association. *J Urol*, 162, 1697-1701.

Solomon, D. A., Kim, J. S., Bondaruk, J., Shariat, S. F., Wang, Z. F., Elkahloun, A. G., Ozawa, T., Gerard, J., Zhuang, D., Zhang, S., Navai, N., Siefker-Radtke, A., Phillips, J. J., Robinson, B. D., Rubin, M. A., Volkmer, B., Hautmann, R., Kufer, R., Hogendoorn, P. C., Netto, G., Theodorescu, D., James, C. D., Czerniak, B., Miettinen, M., & Waldman, T. (2013). Frequent truncating mutations of STAG2 in bladder cancer. *Nat Genet*, 45, 1428-1430.

Steelman, L. S., Navolanic, P. M., Sokolosky, M. L., Taylor, J. R., Lehmann, B. D., Chappell, W. H., Abrams, S. L., Wong, E. W., Stadelman, K. M., Terrian, D. M., Leslie, N. R., Martelli, A. M., Stivala, F., Libra, M., Franklin, R. A., & McCubrey, J. A. (2008). Suppression of PTEN function increases breast cancer chemotherapeutic drug resistance while conferring sensitivity to mTOR inhibitors. *Oncogene*, 27, 4086-4095. doi: 4010.1038/onc.2008.4049. Epub 2008 Mar 4010.

Steelman, L. S., Stadelman, K. M., Chappell, W. H., Horn, S., Basecke, J., Cervello, M., Nicoletti, F., Libra, M., Stivala, F., Martelli, A. M., & McCubrey, J. A. (2008). Akt as a therapeutic target in cancer. *Expert Opin Ther Targets*, 12, 1139-1165. doi: 1110.1517/14728222.14728212.14728229.14721139 .

Stenzl, A., Cowan, N. C., De Santis, M., Kuczyk, M. A., Merseburger, A. S., Ribal, M. J., Sherif, A., & Witjes, J. A. (2011). Treatment of muscle-invasive and metastatic bladder cancer: update of the EAU guidelines. *Eur Urol*, 59, 1009-1018.

Stokoe, D., Stephens, L. R., Copeland, T., Gaffney, P. R., Reese, C. B., Painter, G. F., Holmes, A. B., McCormick, F., & Hawkins, P. T. (1997). Dual role of phosphatidylinositol-3,4,5-trisphosphate in the activation of protein kinase B. *Science*, 277, 567-570.

Tamguney, T., Zhang, C., Fiedler, D., Shokat, K., & Stokoe, D. (2008). Analysis of 3-phosphoinositide-dependent kinase-1 signaling and function in ES cells. *Exp Cell Res*, 314, 2299-2312. doi: 2210.1016/j.yexcr.2008.2204.2006. Epub 2008 Apr 2223.

Tan, D. S., Dumez, H., Olmos, D., Sandhu, S. K., Hoeben, A., Stephens, A. W., Poondru, S., Gedrich, R., Kaye, S. B., & P., S. (2010). First-in-human phase I study exploring three schedules of OSI-027, a novel small molecule TORC1/TORC2 inhibitor, in patients with advanced solid tumors and lymphoma. *J Clin Oncol*, 28, suppl; abstr 3006.

Tanaka, H., Yoshida, M., Tanimura, H., Fujii, T., Sakata, K., Tachibana, Y., Ohwada, J., Ebiike, H., Kuramoto, S., Morita, K., Yoshimura, Y., Yamazaki, T., Ishii, N., Kondoh, O., & Aoki, Y. (2011). The selective class I PI3K inhibitor CH5132799 targets human cancers harboring oncogenic PIK3CA mutations. *Clin Cancer Res*, 17, 3272-3281.

Tazzari, P. L., Cappellini, A., Ricci, F., Evangelisti, C., Papa, V., Grafone, T., Martinelli, G., Conte, R., Cocco, L., McCubrey, J. A., & Martelli, A. M. (2007). Multidrug resistance-associated protein 1 expression is under the control of the phosphoinositide 3 kinase/Akt signal transduction network in human acute myelogenous leukemia blasts. *Leukemia*, 21, 427-438. Epub 2007 Jan 2011.

The Cancer Genome Atlas Research Network,. (2014). Comprehensive molecular characterization of urothelial bladder carcinoma. *Nature*, 2014.

Thoreen, C. C., Kang, S. A., Chang, J. W., Liu, Q., Zhang, J., Gao, Y., Reichling, L. J., Sim, T., Sabatini, D. M., & Gray, N. S. (2009). An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1. *J Biol Chem*, 284, 8023-8032. doi: 8010.1074/jbc.M900301200. Epub 900302009 Jan 900301215.

Tigli, H., Seven, D., Tunc, M., Sanli, O., Basaran, S., Ulutin, T., & Buyru, N. (2013). LKB1 mutations and their correlation with LKB1 and Rheb expression in bladder cancer. *Mol Carcinog*, 52, 660-665.

Tredan, O., Treilleux, I., Wang, Q., Gane, N., Pissaloux, D., Bonnin, N., Petit, T., Cretin, J., Bonichon-Lamichhane, N., Priou, F., Lavau-Denes, S., Mari, V., Freyer, G., Lebrun, D., Alexandre, J., & Ray-Coquard, I. (2013). Predicting everolimus treatment efficacy in patients with advanced endometrial carcinoma: a GINECO group study. *Target Oncol*, 8, 243-251.

Venkatesan, A. M., Chen, Z., dos Santos, O., Dehnhardt, C., Santos, E. D., Ayral-Kaloustian, S., Mallon, R., Hollander, I., Feldberg, L., Lucas, J., Yu, K., Chaudhary, I., & Mansour, T. S. (2010). PKI-179: an orally efficacious dual phosphatidylinositol-3-kinase (PI3K)/mammalian target of rapamycin (mTOR) inhibitor. *Bioorg Med Chem Lett*, 20, 5869-5873.

von der Maase, H., Sengelov, L., Roberts, J. T., Ricci, S., Dogliotti, L., Oliver, T., Moore, M. J., Zimmermann, A., & Arning, M. (2005). Long-term survival results of a randomized trial comparing gemcitabine plus cisplatin, with methotrexate, vinblastine, doxorubicin, plus cisplatin in patients with bladder cancer. *J Clin Oncol.*, 23, 4602-4608.

Wallin, J. J., Edgar, K. A., Guan, J., Berry, M., Prior, W. W., Lee, L., Lesnick, J. D., Lewis, C., Nonomiya, J., Pang, J., Salphati, L., Olivero, A. G., Sutherlin, D. P., O'Brien, C., Spoerke, J. M., Patel, S., Lensun, L., Kassees, R., Ross, L., Lackner, M. R., Sampath, D., Belvin, M., & Friedman, L. S. (2011). GDC-0980 is a novel class I PI3K/mTOR kinase inhibitor with robust activity in cancer models driven by the PI3K pathway. *Mol Cancer Ther*, 10, 2426-2436.

Wang, D. S., Rieger-Christ, K., Latini, J. M., Moinzadeh, A., Stoffel, J., Pezza, J. A., Saini, K., Libertino, J. A., & Summerhayes, I. C. (2000). Molecular analysis of PTEN and MXI1 in primary bladder carcinoma. *Int J Cancer.*, 88, 620-625.

Weisberg, E., Banerji, L., Wright, R. D., Barrett, R., Ray, A., Moreno, D., Catley, L., Jiang, J., Hall-Meyers, E., Sauveur-Michel, M., Stone, R., Galinsky, I., Fox, E., Kung, A. L., & Griffin, J. D. (2008). Potentiation of antileukemic therapies by the dual PI3K/PDK-1 inhibitor, BAG956: effects on BCR-ABL- and mutant FLT3-expressing cells. *Blood.*, 111, 3723-3734. doi: 3710.1182/blood-2007-3709-114454. Epub 112008 Jan 114459.

West, N. W., Garcia-Vargas, A., Chalfant, C. E., & Park, M. A. (2013). OSU-03012 sensitizes breast cancers to lapatinib-induced cell killing: a role for Nck1 but not Nck2. *BMC Cancer*, 13, 1471-2407.

Westhoff, M. A., Kandenwein, J. A., Karl, S., Vellanki, S. H., Braun, V., Eramo, A., Antoniadis, G., Debatin, K. M., & Fulda, S. (2009). The pyridinyfuranopyrimidine inhibitor, PI-103, chemosensitizes glioblastoma cells for apoptosis by inhibiting DNA repair. *Oncogene.*, 28, 3586-3596. doi: 3510.1038/onc.2009.3215. Epub 2009 Jul 3527.

Williams, S. V., Hurst, C. D., & Knowles, M. A. (2013). Oncogenic FGFR3 gene fusions in bladder cancer. *Hum Mol Genet*, 22, 795-803.

Witjes, J. A., Comperat, E., Cowan, N. C., De Santis, M., Gakis, G., Lebre, T., Ribal, M. J., Van der Heijden, A. G., & Sherif, A. (2014). EAU guidelines on muscle-invasive and metastatic bladder cancer: summary of the 2013 guidelines. *Eur Urol*, 65, 778-792.

Wolff, A. C., Lazar, A. A., Bondarenko, I., Garin, A. M., Brinca, S., Chow, L., Sun, Y., Neskovic-Konstantinovic, Z., Guimaraes, R. C., Fumoleau, P., Chan, A., Hachemi, S., Strahs, A., Cincotta, M., Berkenblit, A., Krygowski, M., Kang, L. L., Moore, L., & Hayes, D. F. (2013). Randomized phase III placebo-controlled trial of letrozole plus oral temsirolimus as first-line endocrine therapy in postmenopausal women with locally advanced or metastatic breast cancer. *J Clin Oncol*, 31, 195-202.

Wu, Y. M., Su, F., Kalyana-Sundaram, S., Khazanov, N., Ateeq, B., Cao, X., Lonigro, R. J., Vats, P., Wang, R., Lin, S. F., Cheng, A. J., Kunju, L. P., Siddiqui, J., Tomlins, S. A., Wyngaard, P., Sadis, S., Roychowdhury, S., Hussain, M. H., Feng, F. Y., Zalupski, M. M., Talpaz, M., Pienta, K. J., Rhodes, D. R., Robinson, D. R., & Chinnaiyan, A. M. (2013). Identification of targetable FGFR gene fusions in diverse cancers. *Cancer Discov*, 3, 636-647.

Yaguchi, S., Fukui, Y., Koshimizu, I., Yoshimi, H., Matsuno, T., Gouda, H., Hirono, S., Yamazaki, K., & Yamori, T. (2006). Antitumor activity of ZSTK474, a new phosphatidylinositol 3-kinase inhibitor. *J Natl Cancer Inst.*, 98, 545-556.

Yang, H., Rudge, D. G., Koos, J. D., Vaidialingam, B., Yang, H. J., & Pavletich, N. P. (2013). mTOR kinase structure, mechanism and regulation. *Nature*, 497, 217-223.

Yee, D. S., Ishill, N. M., Lowrance, W. T., Herr, H. W., & Elkin, E. B. (2011). Ethnic differences in bladder cancer survival. *Urology*, 78, 544-549.



You, H., Pellegrini, M., Tsuchihara, K., Yamamoto, K., Hacker, G., Erlacher, M., Villunger, A., & Mak, T. W. (2006). FOXO3a-dependent regulation of Puma in response to cytokine/growth factor withdrawal. *J Exp Med.*, 203, 1657-1663. Epub 2006 Jun 1626.

Yu, K., Shi, C., Toral-Barza, L., Lucas, J., Shor, B., Kim, J. E., Zhang, W. G., Mahoney, R., Gaydos, C., Tardio, L., Kim, S. K., Conant, R., Curran, K., Kaplan, J., Verheijen, J., Ayril-Kaloustian, S., Mansour, T. S., Abraham, R. T., Zask, A., & Gibbons, J. J. (2010). Beyond rapalog therapy: preclinical pharmacology and antitumor activity of WYE-125132, an ATP-competitive and specific inhibitor of mTORC1 and mTORC2. *Cancer Res*, 70, 621-631.

Yu, K., Toral-Barza, L., Shi, C., Zhang, W. G., Lucas, J., Shor, B., Kim, J., Verheijen, J., Curran, K., Malwitz, D. J., Cole, D. C., Ellingboe, J., Ayril-Kaloustian, S., Mansour, T. S., Gibbons, J. J., Abraham, R. T., Nowak, P., & Zask, A. (2009). Biochemical, cellular, and in vivo activity of novel ATP-competitive and selective inhibitors of the mammalian target of rapamycin. *Cancer Res.*, 69, 6232-6240. doi: 6210.1158/0008-5472.CAN-6209-0299. Epub 2009 Jul 6237.

Yuan, J., Mehta, P. P., Yin, M. J., Sun, S., Zou, A., Chen, J., Rafidi, K., Feng, Z., Nickel, J., Engebretsen, J., Hallin, J., Blasina, A., Zhang, E., Nguyen, L., Sun, M., Vogt, P. K., McHarg, A., Cheng, H., Christensen, J. G., Kan, J. L., & Bagrodia, S. (2011). PF-04691502, a potent and selective oral inhibitor of PI3K and mTOR kinases with antitumor activity. *Mol Cancer Ther*, 10, 2189-2199.

Zeng, Z., Samudio, I. J., Zhang, W., Estrov, Z., Pelicano, H., Harris, D., Frolova, O., Hail, N., Jr., Chen, W., Kornblau, S. M., Huang, P., Lu, Y., Mills, G. B., Andreeff, M., & Konopleva, M. (2006). Simultaneous inhibition of PDK1/AKT and Fms-like tyrosine kinase 3 signaling by a small-molecule KP372-1 induces mitochondrial dysfunction and apoptosis in acute myelogenous leukemia. *Cancer Res.*, 66, 3737-3746.

Zeng, Z., Sarbassov dos, D., Samudio, I. J., Yee, K. W., Munsell, M. F., Ellen Jackson, C., Giles, F. J., Sabatini, D. M., Andreeff, M., & Konopleva, M. (2007). Rapamycin derivatives reduce mTORC2 signaling and inhibit AKT activation in AML. *Blood.*, 109, 3509-3512. Epub 2006 Dec 3519.

Zhou, H., & Huang, S. (2011). Role of mTOR signaling in tumor cell motility, invasion and metastasis. *Curr Protein Pept Sci*, 12, 30-42.

Zhu, J., Huang, J. W., Tseng, P. H., Yang, Y. T., Fowble, J., Shiau, C. W., Shaw, Y. J., Kulp, S. K., & Chen, C. S. (2004). From the cyclooxygenase-2 inhibitor celecoxib to a novel class of 3-phosphoinositide-dependent protein kinase-1 inhibitors. *Cancer Res.*, 64, 4309-4318.

Zoncu, R., Efeyan, A., & Sabatini, D. M. (2011). mTOR: from growth signal integration to cancer, diabetes and ageing. *Nat Rev Mol Cell Biol*, 12, 21-35.

Zou, Z. Q., Zhang, L. N., Wang, F., Bellenger, J., Shen, Y. Z., & Zhang, X. H. (2012). The novel dual PI3K/mTOR inhibitor GDC-0941 synergizes with the MEK inhibitor U0126 in non-small cell lung cancer cells. *Mol Med Rep*, 5, 503-508. doi: 510.3892/mmr.2011.3682. Epub 2011 Nov 3816.

**Conflict of Interest:**

NH is the principal investigator of the Phase II trial evaluating efficacy of Temsirolimus in second line therapy for patients with advanced bladder cancer (VESTOR - NCT01827943) sponsored by Pfizer.

PP: None

## Figure Legends

Figure 1: Schematic representation of the PI3K/AKT/mTOR signaling pathway and its cross-talk with the MAPK/MEK/ERK pathway. Activation of receptor tyrosine kinases (RTKs) by their respective ligands activates the recruitment of the PI3Ks and induces the phosphorylation of the p110 subunit that catalyzes the conversion of PIP2 to PIP3. Recruitment of PDK1 to the cell membrane induces AKT phosphorylation that triggers multiple downstream effectors, including TSC1 and TSC2 that inhibit RHEB, a suppressor of mTOR functions. mTOR is part of two separate complexes, mTORC1 and mTORC2. mTORC1 induces the activation of factors involved in the regulation of translation as well as other factors involved in angiogenesis and autophagy. mTORC2 regulates AKT activity by a feed-back loop. The PI3K/AKT/mTOR pathway is regulated by the level of nutrients and shows cross-talks with other pathways regulating cell growth or survival such as the MAPK/MEK/ERK pathway (see Text for details). Interaction of rapamycin with FKBP12 leads to selective inhibition of the mTORC1 complex. Phosphorylations are indicated by red circles with Ps.

Figure 2: Chemical structures of the main PI3K inhibitors

Figure 3: Chemical structures of the main dual inhibitors of PI3K and mTOR

Figure 4: Chemical structures of the main AKT1 inhibitors

Figure 5: Chemical structures of the rapalogs and the main other mTOR inhibitors

Figure 6: Chemical structures of the main PDK1 inhibitors

Gene	Mutations <sup>§</sup>	Frequency <sup>‡</sup> (LOH)	Nb patients	Reference
PIK3CA (p110α)	<b>E545(K,G,Q); E542K; H1047(R,L);</b>	13%	87	(Lopez-Knowles et al., 2006)
	R88Q; C90S; R93W; G106V; E109del; E365K; E417K;	25%	92	(Platt et al., 2009)
	G451(R,V); P471A; E453Q; E493K; Q546(R,L); Q643R;	27%	145	(Sjodahl et al., 2011)
	E726K; G1007R; D1017H; M1043I; G1049(R,S)	24%	144	(Lindgren et al., 2012)
		4.1%	98	(Korkolopoulou et al., 2012)
		34.5%	87	(Duenas et al., 2013)
		18%	95	(Iyer et al., 2013)
		9%	122	(Calderaro et al., 2013)
		19.8%	99	(Guo et al., 2013)
	21%	131	(The Cancer Genome Atlas Research Network, 2014)	
PIK3R1 (p85α)	D19V; E137K; E218*; K288Q; delW237-Y242; R262T; R358*;	< 5%	264	(Ross et al., 2013b) PLoS One
	Q579-Y580del; N377K; N441I; R481W; E518Q; D529N;	<1%	145	(Sjodahl et al., 2011)
	I566-D578del; T645I; R574T	<2%	131	(The Cancer Genome Atlas Research Network, 2014)
PTEN	R11L; R15L; F37S; E214R; R335*; c150fs-indel; c744fs-indel	(39%)	36	(Cappellen et al., 1997)
	<b>LOH (10q23)</b>	(45%)	20	(Kagan et al., 1998)
		8% (23%)	285	(Cairns et al., 1998)
		0% (24.5%)	53	(Aveyard et al., 1999)
		17%	35	(Wang et al., 2000)
		(12%)	92	(Platt et al., 2009)
		0%	145	(Sjodahl et al., 2011)
		(16.5%)	472	(Cordes et al., 2013)
		4%	95	(Iyer et al., 2013)
		(40%)	122	(Calderaro et al., 2013)
	3% (13%)	131	(The Cancer Genome Atlas Research Network, 2014)	
AKT1	<b>E17K;</b>	2.7%	184	(Askham et al., 2010)
	Q214R; D248H; D323G; c150fs-indel; c744fs-indel	<2%	145	(Sjodahl et al., 2011)
		3%	98	(Korkolopoulou et al., 2012)
		2.1%	97	(Iyer et al., 2013)
		<5%	99	(Guo et al., 2013)
LKB1	<b>S19*</b> ; L102Q	76%	50	(Tigli et al., 2013)
RHEB	Y35C	<1%	99	(Guo et al., 2013)
TSC1	S35C; Y49*; Q55*; H58R; H68R; H105R; F158C; S183N;	11% (50%)	62	(Knowles et al., 2003)
	Y185(C,*); R190P; Y192C; L203S; H206D; F216A; V220F;	14.5%	154	(Pymar et al., 2008)
	Q328*; W347*; S403L; T417I; T441*; K477*; E478*;	16%(40%)	92	(Platt et al., 2009)
	R484G; E493K; D504N; R509*; Q516*; R517*; L536*; L576C;	11.7%	145	(Sjodahl et al., 2011)
	E577*; E609Q; E636fs; R692*; Q694*; K737Splice; Q739*;	8%	110	(Iyer et al., 2012)
	R786Q; V858L; S871*; S1025G; c492fs-indel; c595fs-indel;	5%	99	(Guo et al., 2013)
	c1257fs-indel; c1958fs-indel;	7%	95	(Iyer et al., 2013)
	<b>LOH (9q34)</b>	5% (54%)	41	(Guo et al., 2013b)
	8%	131	(The Cancer Genome Atlas Research Network, 2014)	
TSC2	L423F; G1494D; Y1186C; H1727N; E1805G; I173fs;	(15%)	92	(Platt et al., 2009)
	<b>LOH (16p13)</b>	3%	145	(Sjodahl et al., 2011)
		<1% (32%)	99	(Guo et al., 2013)
		<2%	131	(The Cancer Genome Atlas Research Network, 2014)

**Table 1:** Mutations of the genes involved in the PI3K/AKT/mTOR signaling pathway found in urothelial bladder cancers. <sup>§</sup> The most frequent mutations and/or LOH are indicated in bold; indel: insertion or delation mutations; fs: frame-shift. \* Missense mutations leading to early termination codon. <sup>‡</sup> Percentages in parentheses indicate the percentage of LOH for the corresponding gene.

Drugs	Target	Indication	Phase	Reference*
Wortmannin	PI3K, (mTOR, DNA-PK, MAPK)		Preclinical	
PWT-458	PI3K, (mTOR, DNA-PK, MAPK)		Preclinical	
PX-866	PI3K	mCRPC, SCCHN, Glioblastoma, B-RAF mutant tumors	I/II	(Bowles et al., 2013)
SF-1126	PI3K, (mTOR)	Advanced or metastatic solid tumors	I	NCT00907205
GDC-0941 (Pictilisib)	PI3K $\alpha$ and $\delta$ , (HIF1 $\alpha$ )	Metastatic Breast cancer, NSCLC, Non-Hodgkin's Lymphoma, Solid Cancers	I/II	<a href="http://clinicaltrials.gov">http://clinicaltrials.gov</a>
GDC-0032	PI3K	Solid tumors	I	NCT01296555, NCT01862081
CAL-101 (Idelalisib)	PI3K $\delta$	Chronic Lymphocytic Leukemia	III	(Furman et al., 2014)
XL-147		Solid tumors, Endometrial cancers	I/II	(Shapiro et al., 2014), NCT01013324
BAY 80-6946 (Capanisib)	PI3K $\alpha$ and $\delta$	Solid tumors, Non-Hodgkin's Lymphoma	I/II	<a href="http://clinicaltrials.gov">http://clinicaltrials.gov</a>
BYL719	PI3K $\alpha$	Advanced solid tumors & PIK3CA-mutated tumors	I/II	<a href="http://clinicaltrials.gov">http://clinicaltrials.gov</a>
BKM120 (Buparlisib)	PI3K	Advanced or Metastatic Breast Cancer	III	NCT01572727, NCT01633060, NCT01610284
GSK-2636771	PI3K $\beta$	Advanced solid tumors with PTEN deficiency	I	NCT01458067
TGX-221	PI3K $\beta$		Preclinical	
AS-252424	PI3K $\gamma$		Preclinical	
CH5132799	PI3K	Solid tumors	I	NCT01222546
ZSTK474	PI3K	Advanced solid tumors	I	NCT01682473, NCT01280487
GS-9820	PI3K $\delta$	Lymphoid malignancies	I	NCT01705847
PI-103	PI3K, mTOR, (DNA-PK)	Preclinical	Preclinical	
WJD008	PI3K $\alpha$ , mTOR	Preclinical	Preclinical	
XL-765	PI3K, mTOR	Breast, NSCLC, Glioblastomas, Solid tumors	I/II	<a href="http://clinicaltrials.gov">http://clinicaltrials.gov</a>
GSK2126458	PI3K, mTOR	Refractory solid tumors	I	(Munster et al., 2012)
GSK-1059615	PI3K, mTOR	Solid tumors, Lymphoma	I	NCT00695448
GDC-0980	PI3K, mTOR	Endometrial carcinoma, Breast, Prostate, Renal carcinoma	II	<a href="http://clinicaltrials.gov">http://clinicaltrials.gov</a>
GNE-477	PI3K, mTOR		Preclinical	
NVP-BE2235	PI3K, mTOR	Advanced solid tumors	II	<a href="http://clinicaltrials.gov">http://clinicaltrials.gov</a>
NVP-BGT226	PI3K, mTOR	Advanced solid tumors	I	(Markman et al., 2012)
PKI-179	PI3K, mTOR	Advanced solid tumors	I	NCT00997360
PKI-402	PI3K, mTOR		Preclinical	
PF-05212384 (PKI-587)	PI3K, mTOR	Metastatic colorectal cancer	II	NCT01925274
PF-04691502	PI3K, mTOR	Solid tumors	I	(Britten et al., 2014)
Perifosine	AKT	Advanced colorectal cancer, Multiple myeloma	III	NCT01097018, NCT01002248
MK-2206	AKT	Solid tumors, leukemias	II	<a href="http://clinicaltrials.gov">http://clinicaltrials.gov</a>
Triciribine	AKT	Breast, Ovarian cancers, Leukemia, metastatic cancers	I	<a href="http://clinicaltrials.gov">http://clinicaltrials.gov</a>
A-443654	AKT		Preclinical	
GDC-0068	AKT	mCRPC, Advanced Gastric cancers	II	NCT01485861, NCT01896531
XL-418	AKT, (p70S6K)	Solid tumors	I	NCT00460278
GSK-690693	AKT	Hematologic malignancies	I	NCT00666081, NCT00493818
AZD-5363	AKT	mCRPC, Breast, NSCLC	II	NCT02121639, NCT02077569, NCT02117167
KP372-1	AKT, PDK1		Preclinical	
PP-242	mTORC1/mTORC2		Preclinical	
INK-128 (MLN0128)	mTORC1/mTORC2	Advanced solid tumors,	I	<a href="http://clinicaltrials.gov">http://clinicaltrials.gov</a>
OSI-027	mTORC1/mTORC2	Advanced solid tumors, lymphoma	I	(Tan et al., 2010)
OXA-01	mTORC1/mTORC2, (AKT, p70S6K)		Preclinical	
Palomid 529	mTORC1/mTORC2		Preclinical	
Ku0063794	mTORC1/mTORC2, p70S6K		Preclinical	
AZD-8055	mTORC1/mTORC2	Advanced solid tumors	I	(Asahina et al., 2013)
AZD-2014	mTORC1/mTORC2	Metastatic renal cancer, Metastatic NSCLC	II	NCT01793636, NCT02117167
WAY-600	mTORC1/mTORC2		Preclinical	
WYE-687	mTORC1/mTORC2		Preclinical	
WYE-132	mTORC1/mTORC2		Preclinical	
WYE-354	mTORC1/mTORC2		Preclinical	
Torin1	mTORC1/mTORC2		Preclinical	
Torin2	mTORC1/mTORC2, ATM, ATR, DNA-PK		Preclinical	
Sirolimus	mTORC1	Solid tumors	II	<a href="http://clinicaltrials.gov">http://clinicaltrials.gov</a>
Everolimus	mTORC1	Advanced gastric cancer, Breast cancers, Advanced lung cancer, Astrocytoma, Neuroendocrine tumors, mRCC	III	(Ohtsu et al., 2013), (Campane et al., 2013), (Fazio et al., 2013), (Franz et al., 2013), (Pavel et al., 2011), (Motzer et al., 2010)
Temsirolimus	mTORC1	mRCC, Metastatic breast cancer, Mantel cell lymphoma	III	(Hutson et al., 2014), (Rini et al., 2014), (Wolff et al., 2013), (Hess et al., 2009)
Ridaforolimus	mTORC1	Metastatic sarcomas	III	(Demetri et al., 2013)
NVP-BAG956	PDK1, PI3K		Preclinical	
Celecoxib	PDK1, COX2	Metastatic colorectal cancer, NSCLC, Prostate cancer, Breast	III	NCT00064181, (Groen et al., 2011), (James et al., 2012), (Chow et al., 2013)
OSU-03012	PDK1		Preclinical	
GSK-2334470	PDK1		Preclinical	
BX-795	PDK1, ERK8, TBK1, IKK- $\epsilon$		Preclinical	
BX-912	PDK1		Preclinical	
BX-320	PDK1		Preclinical	

Table 2: Inhibitors of the PI3K/AKT/mTOR signaling pathway with the latest status of their development in the treatment of cancers. Clinical trials in bladder cancers are not included and are detailed in Table 3. (\*) The status of ongoing trials refers to the NCT number indicated on the National Institutes of Health ClinicalTrials.gov website. RCC: Renal Cell Carcinoma; NSCLC: Non-Small Cell Lung Cancer; SCCHN: Small Cell carcinoma of the Head & Neck; mCRPC: metastatic Castration-Resistant Prostate Cancer

Drugs	Phase	Indication	Nb Patients	Results/Status	Reference*
Everolimus	II	Post Cisplatin	45	PFS: 2.6 months OS: 8.3 months	(Milowski et al., 2013)
Everolimus	II	Post Cisplatin	37	PFS: 2 months OS: 3.4 months	(Seront et al., 2012)
Temsirolimus	II	Second line	15	PFS: 2.5 months OS: 3.5 months	(Gerullis et al., 2012)
Sirolimus	0	Neoadjuvant bladder cancer		Recruiting	NCT01827618
Sirolimus + Cisplatin + Gemcitabine	I/II	First line advanced bladder cancer		Recruiting	NCT01938573
Temsirolimus + Cisplatin + Gemcitabine	I/II	First line metastatic bladder cancer	99	Completed	NCT01090466
Temsirolimus	II	Second line metastatic bladder cancer		Recruiting	NCT01827943
Everolimus	II	Post Cisplatin	40	Completed	NCT00714025
Everolimus	II	Second line metastatic bladder cancer		Active not recruiting	NCT00805129
Everolimus + Gemcitabine + Cisplatin (Split I	I	Advanced solid tumors (bladder, kidney, ureter)		Recruiting	NCT01182168
Everolimus + Paclitaxel	II	First line for unfit patients for cisplatin		Recruiting	NCT01215136
Everolimus + Paclitaxel	II	Second line metastatic bladder cancer		Terminated	NCT00933374
BKM120 (Buparlisib)	II	Second line metastatic bladder cancer		Recruiting	NCT01551030
Celecoxib	II/III	Recurrent bladder cancer	152	Completed	NCT00006124
GSK2126458	I	Advanced solid tumors	170	Closed	(Munster et al., 2012)

Table 3: Clinical trials with inhibitors of the PI3K/AKT/mTOR signaling pathway in bladder cancers. For published trials, results show median progression free survival (PFS) and overall survival (OS) in months. (\*) The status of ongoing trials refers to the NCT number indicated on the National Institutes of Health ClinicalTrial.gov website.