

Phosphatidylinositol 3-kinase-AKT-mammalian target of rapamycin (PI3K-Akt-mTOR) signaling pathway in non-small cell lung cancer

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Abstract: Non-small cell lung cancer (NSCLC) is a devastating disease with poor prognosis. Systemic chemotherapy has been the mainstay of treatment in advanced disease for many decades. Personalized targeted therapy such as epidermal growth factor receptor tyrosine kinase inhibitors (EGFR TKIs) and crizotinib has significantly changed the treatment paradigm in NSCLC. The future success of development of molecular targeted therapy relies on the understanding of signal transduction pathways. The PI3K-Akt-mTOR pathway is commonly deregulated in human malignancy including NSCLC. Therefore, this pathway is a target for many therapeutic developments. This review will provide an overview of PI3K-Akt-mTOR signaling pathway, genetic alterations activating the pathway and clinical therapeutic development of pathway inhibitors.

Keywords: PI3K-Akt-mTOR pathway; non-small cell lung cancer (NSCLC)

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Introduction

Lung cancer is the most common cancer and leading cause of cancer death worldwide, with an incidence of 1.6 million new cases annually and 1.38 million deaths in 2008 (1). It is the fifth most common cancer and the leading cause of cancer death in Australia. There are approximately 9,700 new cases of lung cancer diagnosed each year. In 2007, there were 7,626 deaths from lung cancer in Australia, accounting for 19% of all cancer deaths (2,3).

For the past two decades, decisions regarding lung cancer treatment have been based largely on the histological distinction between non-small cell lung cancer (NSCLC) and small cell lung carcinoma. In recent years, more definitive histological classifications as well as identification of somatic mutations have become an essential component in determining the management of NSCLC. Molecular driven therapeutic targets such as *epidermal growth factor receptor (EGFR)* gene mutations (4) and abnormal fusion of *echinoderm microtubule-associated protein-like 4* and

anaplastic lymphoma kinase (EML4-ALK) genes (5) have resulted in a paradigm shift in the treatment of advanced lung adenocarcinoma. However, they only account for a small proportion of NSCLC. Therefore, there are ongoing efforts in identifying more molecular targets for potential targeted therapeutics. *EGFR* mutation and abnormal fusion of *EML4-ALK* activate two main downstream signaling pathways, RAS-RAF-MEK-ERK and PI3K-Akt-mTOR, resulting in uncontrolled growth and cell proliferation. PI3K-Akt-mTOR is one of the most commonly deregulated pathways (6), which has been implicated in the tumorigenesis of NSCLC. Therefore, there has been an increasing research interest in identifying novel therapies to target this signaling pathway.

This review is going to provide an overview of the biology of PI3K-Akt-mTOR signaling pathway in normal cells under physiological conditions, mechanisms of deregulation of the pathway in NSCLC, therapeutic implications and potential prognostic and predictive biomarkers of the PI3K-Akt-mTOR signaling pathway.

Biology of PI3K-Akt-mTOR pathway

Key components of PI3K-Akt-mTOR pathway

PI3Ks are a family of lipid kinases that phosphorylate the 3'-hydroxyl group in phosphatidylinositol and phosphoinositides (7). There are three classes of PI3K (I-III), which are classified according to their structure and substrate specificity (8). They are heterodimeric proteins with catalytic and regulatory subunits. Class I divides into Class IA and IB based on the types of receptors that they are activated by and they consist of different catalytic and regulatory subunits, each has different isoforms. Class IA PI3Ks consist of a p110 catalytic subunit and a p85 regulatory subunit. They are activated by growth factor receptor tyrosine kinases (RTKs). The p110 catalytic subunit has three isoforms (p110 α , p110 β and p110 δ) that are encoded by *PIK3CA*, *PIK3CB* and *PIK3CD* genes respectively. The p85 regulatory subunit has five isoforms (p50 α , p55 α , p85 α , p85 β and p55 γ). The p85 α , p50 α and p55 α are encoded by *PIK3R1* gene; p85 β encodes for *PIK3R2* gene and p55 γ encodes for the *PIK3R3* gene. Class IB PI3Ks consist of a p110 γ catalytic subunit and a p101 regulatory subunit. They are activated by G-protein coupled receptors (GPCRs) (7,9). The role of Class IA PI3Ks in carcinogenesis has been well demonstrated but Class IB PI3Ks are less clear (10). Class II PI3Ks has only a single p110-like catalytic subunit that catalyze the production of PtdIns[3]P and PtdIns[3,4]P₂ and regulate clathrin mediated membrane trafficking (8,11). Class III consists of a single member, hVPS34 which produces PtdIns[3]P and is involved in the regulation of vesicle trafficking, activation of mTOR by amino acids and autophagy (12,13).

Akt, is a serine/threonine-specific protein kinase and also known as protein kinase B (PKB). It consists of an amino-terminal pleckstrin homology (PH) domain, a central catalytic domain and a short carboxy-terminal regulatory domain. There are three isoforms: Akt 1 (PKB α), Akt 2 (PKB β) and Akt 3 (PKB γ) (10). The function of Akt is to phosphorylate and activate or inactivate numerous downstream cytoplasmic and nuclear substrates such as forkhead (FOXO) family of transcription factors, p-53 binding protein (MDM2), pro-apoptotic protein BCL2-antagonist of cell death (BAD) and tuberous sclerosis 1 and 2 (TSC1 & 2) to regulate cell survival, proliferation and protein synthesis, and hence cell growth (7,10).

The mammalian target of rapamycin (mTOR) is a serine-threonine kinase that is a member of the phosphatidylinositol kinase-related kinase (Pik) family

of kinases (9). It presents in two multi-protein complexes (mTORC1 and mTORC2). The mTORC1 is an mTOR complex combining regulatory-associated protein of mTOR (Raptor), PRAS40 (also known as Akt substrate 1) and mLST8 while mTORC2 combines with rapamycin-insensitive companion of mTOR (Rictor), mSIN1, Protor and mLST8. It carries out its function of control on cell growth and division as well as protein translation through pathways of ribosomal p70 S6 kinase (p70S6K) and the eukaryotic translation initiation factor 4E (eIF4E) binding proteins (4E-BPs) (14).

PI3K-Akt-mTOR signaling in normal cells under physiological condition

Physiologically, the PI3K-Akt-mTOR pathway is activated by the binding of the ligand to the tyrosine kinase receptors (RTKs) such as EGFR, ErbB3, MET, PDGFR, VEGFR, IGF-1R, HER2/neu resulting in recruitment of class IA PI3Ks to the cell membrane where they convert phosphatidylinositol-4,5-bisphosphate (PIP₂) to phosphatidylinositol-3,4,5-trisphosphate (PIP₃). Simultaneously, the Ras-Raf-MAPK pathway may also be activated leading to cross-talk with PI3K. The PIP₃ generated at the cell membrane acts as a second messenger, which binds to the PH domain of Akt. Akt is then phosphorylated at Thr308 in the catalytic domain by the phosphoinositide dependent kinase 1 (PDK1) and at Ser473 in the C-terminal hydrophobic motif by the mammalian target of rapamycin complex 2 (mTORC2). This results in full activation of Akt that lead to phosphorylation and inactivation of the complex of tuberous sclerosis 2 and 1 (TSC2-TSC1), which is a GTPase-activating protein (GAP) for Ras homologue enriched in brain (RHEB). ERK-Rsk also inactivates TSC2. Both results in accumulation of GTP-bound RHEB, leading to activation of the mammalian target of rapamycin complex 1 (mTORC1), which phosphorylates p70 S6 kinase and 4E-binding protein 1 (4EBP1), 4EBP2 and 4EBP3 resulting increased protein translation, ribosome biogenesis and inhibition of autophagy (15-19) (*Figure 1*).

Phosphorylation of Akt not only activates the mTOR signaling but it also activates minute double minute 2 (MDM2) and inactivates targets for pro-apoptotic proteins such as forkhead box O (FOXO) belong to forkhead family of transcription factors and BAD, which are involved in cell survival (*Figure 1*). Akt phosphorylates FOXO resulting in activation of pro-apoptotic proteins such as BIM and FAS

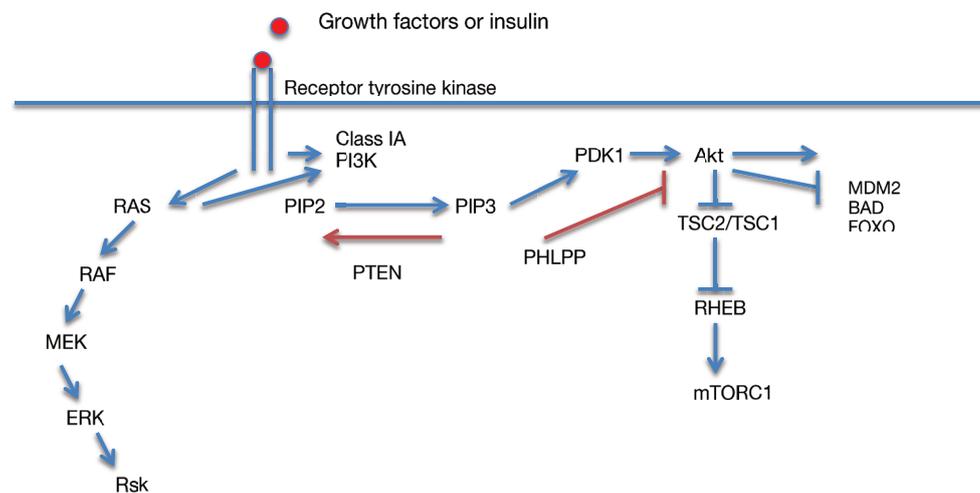


Figure 1 PI3K-Akt-mTOR signaling pathway.

ligand and expression of p27Kip1 and retinoblastoma-like 2 (RBL2) causing cell cycle arrest (20). There are a number of review articles that have elegantly illustrated and described this complex network of downstream substrates of Akt (7,8,10,14).

Feedback and regulation mechanisms of PI3K-Akt-mTOR signaling

In order to maintain cellular homeostasis, there are a number of different mechanisms that regulate the PI3K-Akt-mTOR signal transduction pathway preventing abnormal growth and cell division. Phosphatase and tensin homolog (PTEN) is the key component involved in deactivation of PI3K signaling by converting PIP3 back to PIP2 at the cell membrane (21) while the phosphatase PHLPP inactivates Akt signaling by dephosphorylating Akt at Ser 473 (22) (Figure 1). When it comes to the regulation of mTOR signaling, it is directly influenced by the activity of TSC2-TSC1 complex, which itself is controlled by the activity of TSC2-TSC1 complex, which itself is controlled by the cellular energy level via LKB1 (liver kinase b1)/STK11-AMPK (5'adenosine monophosphate-activated protein kinase) pathway (23), cellular oxygenation via hypoxia-inducible factor- α (HIF α) and DNA damage response 1 (REDD1) (24) and amino acid availability mediated by class III PI3K (25,26). Furthermore, there is an inhibitor protein, PRAS40 within the mTORC1 that exerts control on mTOR signaling. Carracedo *et al.* (27) illustrated that the feedback and regulation mechanisms of PI3K-Akt-mTOR are far more complex.

Deregulation of PI3K-Akt-mTOR signal transduction in NSCLC

Hanahan and Weinberg (28) described that tumourigenesis is the result of the ability of cancer cells to sustain proliferative signaling, evade growth suppressors, activate invasion and metastasis, enable replicative immortality, induce angiogenesis and resist cell death. Many studies have demonstrated that there is 50-70% overexpression of phosphorylated Akt in NSCLC indicating that abnormal activation of PI3K-Akt-mTOR pathway is a frequent event (29-31). Constitutive activation of PI3K-Akt-mTOR signaling pathway could be the result of genetic aberrations in any components of PI3K-Akt-mTOR pathway, its negative regulators, interconnected pathways and RTK signaling resulting in abnormal growth and cell proliferation. *EGFR* mutation and increased copy numbers, *MET* mutation and amplification and *EML4-ALK* rearrangement are examples of genetic changes that could result in abnormal RTK signaling. *EGFR* mutations are more prevalent in East Asian (up to 60%) than Caucasian patients (15-20%) (32-36). *EML4-ALK* rearrangement is much less prevalent (2-7%) and does not have an ethnicity predilection (37,38). However, both driver mutations are more common in patients who are non-smokers than smokers. At the PI3K-Akt-mTOR pathway, *PIK3CA* mutation and amplification, *PTEN* loss, *Akt1* and *LKB1* mutation are examples of genetic abnormalities involved in deregulation of signal transduction (6-8). Furthermore, Ras-Raf-MEK-ERK-Rsk pathway cross talks with PI3K-

Akt-mTOR, therefore, *KRAS* mutations also play a role (39). Some of these genetic changes are more prevalent in certain histological subtypes of NSCLC than others. *EGFR*, *KRAS* mutations and *EML4-ALK* rearrangement are more common in adenocarcinoma than squamous cell carcinoma (32,33,35-38,40,41) while *PIK3CA* amplification is more common in squamous cell carcinoma than non-squamous cell carcinoma (42-48). *PTEN* loss is equally common in both NSCLC with squamous and non-squamous histology (33,49,50). In adenocarcinoma, *EGFR*, *KRAS* mutations and *EML4-ALK* rearrangement are mutually exclusive while *KRAS* and *PIK3CA* co-mutations are common (51). *LKB1* mutations are more common in NSCLC with non-squamous cell histology than squamous and often present with *EGFR* and/or *KRAS* mutations (52-56).

Therapeutic implications

From the RTKs signaling to PI3K-Akt-mTOR pathway, these are targets of major therapeutics development that are either already in clinical practice such as epidermal growth factor tyrosine kinase inhibitors, EGFR TKIs (gefitinib, erlotinib and afatinib) and mTOR inhibitors (everolimus and temsirolimus) or in development such as dual PI3K/mTOR, PI3K and Akt inhibitors.

Targeted therapy—approved for clinical use

EGFR TKIs are now the first line treatment for *EGFR* mutated advanced NSCLC of non-squamous histology. In 2009, Mok *et al.* (36) demonstrated in the Iressa Pan-Asia Study (IPASS) that patients treated with efatinib whose pulmonary adenocarcinoma harbored *EGFR* mutation have a longer progression-free survival than those who were treated with carboplatin and paclitaxel (HR 0.48; 95% CI: 0.36-0.64; $P < 0.001$) in East Asia. Subsequent clinical trials with the same design using erlotinib or afatinib including Asian or western population reached the same conclusion (57-59). Crizotinib, an oral tyrosine kinase inhibitor targeting *ALK* (60), *MET* (61) and *ROS1* (62) has also been approved in advanced non-squamous cell lung carcinoma with *ALK* translocation. Shaw *et al.* (63) demonstrated that crizotinib improved both progression-free survival (PFS) (median 7.7 *vs.* 3 months; HR 0.49; $P < 0.001$) and overall response rate (65% *vs.* 20%; $P < 0.001$) in patients with *ALK* positive non-squamous cell lung carcinoma who progressed after platinum-doublet chemotherapy. Similar benefit when use in the first line setting among patients

with *ALK* positive non-squamous cell lung carcinoma (64). mTOR inhibitors have been approved for clinical use as monotherapy in advanced renal cell carcinoma, pancreatic neuroendocrine tumour and in combination with exemestane in estrogen receptor positive, HER2-negative breast cancer but not in advanced NSCLC because of poor response rate with increased toxicities. Soria *et al.* (65) showed that the overall response rate of everolimus monotherapy was less than 5% in patients with advanced NSCLC who progressed from chemotherapy. Ramalingam *et al.* (66) demonstrated that only 2 out of 28 (7%) patients had a partial response with the combination of everolimus with docetaxel while Besse *et al.* (67) failed to meet the predefined clinically meaningful treatment benefit threshold of 15% or greater with the combination of everolimus with erlotinib over erlotinib alone in the 3-month disease control rate but increased toxicities.

Targeted therapy—under development

Inhibitors targeting the key components of PI3K-Akt-mTOR pathway involving NSCLC are all undergoing either phase I or II therapeutic development. All these novel agents are administered orally. Their toxicities are unique to the components of the pathway that they are blocking but the common side effects include fatigue, anorexia, rash, hyperglycaemia, nausea and diarrhea (68).

PI3K inhibitors

Wortmannin and its derivative LY294002 were the first generation pan-PI3K inhibitors but they have not been moved into clinical use because of their toxicities. Pan-PI3K and isoform specific PI3K inhibitors are the two types of PI3K inhibitors that are in clinical development. Pan-PI3K inhibitors inhibit all isoform of class IA PI3K while isoform-specific PI3K inhibitors inhibit the specific isoforms of catalytic subunit of class IA PI3K (p110 α , p110 β and p110 δ). BKM120 (Buparlisib), GDC-0941 (Pictilisib), PX-866 and XL-147 (SAR 245408) are examples of pan-PI3K inhibitors. BYI719 is an example of isoform-specific PI3K inhibitor.

Buparlisib (BKM120) is an oral pyrimidine-derived pan-PI3K inhibitor against both wild-type and mutant class I PI3Ks isoforms. It does not inhibit the class III PI3K or Mtor (69). The maximal tolerated dose has been established in phase I clinical trials with most common adverse events including rash, hyperglycaemia, anorexia, nausea and diarrhea and there was no ethnic difference in pharmacokinetics properties (70-72). It has advanced the

furthest in the therapeutic development among all PI3K inhibitors as it is being evaluated in a randomised placebo-controlled phase III clinical trial in receptor positive but HER2 negative breast cancer (BKM120 + placebo versus BKM120 + fulvestrant) (73). However, buparlisib is still undergoing early phases of development in NSCLC.

Akt inhibitors

Their mode of action is to block the serine/threonine kinase Akt but their disturbance to the metabolic homeostasis causing severe hyperglycaemia and other potential metabolic abnormalities could hamper the development of this group of agents (74-76). MK-2206 is an example of a pan-Akt kinase inhibitor, which has been shown to potentiate the pathway inhibition when combined with systemic chemotherapy or molecularly targeted agents in preclinical study (77). Therefore, it is being evaluated in a phase II study in combination with erlotinib for patients who have advanced NSCLC after progression from erlotinib (NCT01294306).

Dual PI3K-mTOR inhibitors

SAR245409 and BEZ235 are two examples of dual PI3K-mTOR inhibitors. SAR245409 is a selective inhibitor of Class I PI3Ks, TORC1 and TORC2 (78) and BEZ235 is an imidazo-quinoline derivative blocking the activity of both PI3K and mTOR simultaneously (69,79). Both have been demonstrated to have anti-tumour effect in preclinical studies (80,81) and are being evaluated in early phases of clinical trials in combination with another targeted agents (69,78).

Trials in progress

All the novel agents targeting the PI3K-Akt-mTOR pathway mentioned above are still undergoing early phases of development in NSCLC. Hypotheses generated from the preclinical models, suggested that PI3K pathway inhibitors have the ability to overcome RTK resistance (77,82,83) and they have synergistic effect when combined with cytotoxic chemotherapy (42) or other targeted agents of interconnected pathways such as MEK inhibitors (69,84). Therefore, combination therapeutics approach would yield more success than monotherapy (85). Lockwood *et al.* (86) demonstrated in their genomic study that the genetic pathways involved in squamous cell lung carcinoma are different from those in non-squamous. NOTCH3 and FOXM1 are overexpressed in squamous cell lung carcinoma and may be involved in cross-talk with PI3K pathway

(86-88). This highlighted the importance of histology. Therefore, not only do we need to evaluate markers by genetic alternations but also by histology subtype. Clinical trials in progress that evaluate the efficacy of PI3K pathway inhibitors in advanced NSCLC below include biomarker analysis by histology subtype (squamous cell carcinoma versus non-squamous cell carcinoma) and/or enriched with *PIK3CA*, *PTEN* gene mutated tumours.

PI3K pathway inhibitors + EGFR TKI

Ultimately, all patients with *EGFR* mutated lung adenocarcinoma who are on first generation EGFR TKIs (gefitinib and erlotinib), will eventually develop resistance and cancer progression. The mechanisms of resistance include acquired resistance mutation *T790M*, *MET* amplification or *PIK3CA* mutation resulting in activation of PI3K signaling. *PIK3CA* mutation account for up to 5% of EGFR-mutated NSCLC acquired resistance to EGFR TKI (89). Studies have demonstrated that adding PI3K inhibitors to EGFR TKIs can overcome the EGFR TKI resistant NSCLC cell lines (82,83,90,91). Tan *et al.* (92) demonstrated in a phase Ib study of 15 patients with *EGFR* mutant NSCLC who progressed during or after gefitinib that it was safe to combine buparlisib 80 mg with gefitinib 250 mg and there was antitumour activity but significant toxicities with 40% of patients experiencing delayed grade 3 rash and diarrhea. The median progression free survival was 2.8 (range, 2.3-8.1) months. Four out of nine patients in the group that included patients who progressed while on gefitinib had clinical responses including slight tumor shrinkage and reduced pleural effusion. Molecular analysis showed 6 (50%) of 12 patients whose tumors harbored *T790M* mutation, 2 (40%) of 5 had *MET* amplification while no patients whose tumor had *PIK3CA* mutation or *PTEN* loss. The dose expansion phase and alternate dose schedule are ongoing. Another similar phase Ib/II trial but in combination with erlotinib is currently recruiting (NCT01487265).

PI3K pathway inhibitors + chemotherapy

BASALT-1 (NCT01297491) is a two-stage phase II trial that included patients with metastatic NSCLC and PI3K pathway activation defined by *PIK3CA* mutation, *PTEN* mutation or loss of *PTEN* expression by immunohistochemistry. All patients in the first stage of the trial received buparlisib as a single agent and were stratified into squamous and non-squamous NSCLC subgroups. In the second stage, it was

planned to randomise patients with squamous NSCLC to either buparlisib (100 mg/day) or docetaxel (75 mg/m² every 3 weeks) while patients with non-squamous NSCLC randomise to either buparlisib (100 mg/day) or docetaxel (75 mg/m²) or pemetrexed (500 mg/m²) every three weeks. However, Soria *et al.* (93) demonstrated the progression free survival rate at 12 weeks was less than 50% for both the squamous and non-squamous NSCLC groups. Therefore, the second stage was not initiated because of futility. BASALT-2 (NCT01820325) is a phase Ib/II trial to determine the safety and efficacy of buparlisib in combination with carboplatin (area under the curve, AUC 6) and paclitaxel (200 mg/m²) every 3 weeks as first line treatment for patients with squamous NSCLC. NCT01911325 is another phase Ib/II trial that includes only squamous NSCLC.

There are other phase I or II trials using different chemotherapy regimens with buparlisib. Carboplatin with pemetrexed (NCT01723800 is recruiting) and cisplatin with gemcitabine are the two regimens used in two phase I trials and one has not opened yet (NCT01971489). Besse *et al.* (94) demonstrated in a phase Ib study that included patients with stage IIIB/IV NSCLC who failed first or second line chemotherapy that the overall response rate for squamous NSCLC patients was 75% (one complete responder and 2 partial responders) while non-squamous NSCLC eligible for bevacizumab was 66% (6 partial responders). There were no dose-limiting toxicities at 250 mg and the maximum dose for evaluation was at 330 mg. In the study, patients were stratified into two groups based on histology. For patients with squamous histology and the lesions centrally located or those with non-squamous histology and had contraindications to bevacizumab such as hypertension, haemoptysis or haematuria, pictilisib and carboplatin with paclitaxel were given for six cycles followed by maintenance pictilisib until progression (Arm A). For patients with non-squamous histology or squamous histology of peripherally locating lesions and no contraindications to bevacizumab, six cycles of carboplatin with paclitaxel and bevacizumab and pictilisib was given followed by maintenance bevacizumab with pictilisib (Arm B).

Prognostic and predictive biomarkers in NSCLC

Prognostic factor refers to patient or cancer characteristics that identify patients who are going to have a better or poorer survival regardless of treatment. They are validated in retrospective studies and are more important in early stage NSCLC because patients identified as high-risk may

benefit from additional adjuvant therapies. Predictive factor refers to patient or cancer characteristics that identify patients who are more likely to respond to a particular treatment. They are validated in randomized controlled trials that can examine the predictive effect of the marker in both the placebo and treatment groups. They are more important in the advanced stage NSCLC because this allows delivery of personalised medicine to patients by maximizing benefit while minimizing potential harm. Genes, proteins, mRNAs and miRNAs are potential biomarkers, which can be discovered and evaluated using many techniques including immunohistochemistry, multi-gene profiling or next generations sequencing. With the advancement of molecular technology, we are increasingly more likely to classify and treat NSCLC according to their molecular genetic aberrations such as using EGFR TKIs and crizotinib in *EGFR* and *ALK* mutant NSCLC respectively. However, they only account for and benefit a small proportion of patients with advanced NSCLC. Furthermore, the clinical activity of novel targeted inhibitors targeting the PI3K-Akt-mTOR pathway, have been modest. Therefore, it would likely be cost ineffective to treat all patients with them. It is important to identify predictive biomarkers that could select patients who are more likely to respond to the novel targeted therapies than those who do not. To date, *EGFR* and *ALK* are the only predictive biomarkers in advanced NSCLC. There have been no predictive markers identified that correlate with clinical activity of novel targeted PI3K, Akt or dual PI3K/mTOR agents yet despite the effort of evaluating tumour molecular status in early phases of therapeutic clinical trials. In a preliminary analysis of a phase I buparlisib dose-escalation trial of 35 patients, over 70% of patients had adequate tumor to evaluate for *PIK3CA* status, PTEN expression and *KRAS* status (70). The study included only 2 patients (5.7%) with lung cancer and the majority of patients had colorectal (43%) or breast cancers (26%). There was one patient with triple-negative breast cancer who showed partial response while seven patients (20%) had stable disease of eight months or more. Five of them had tumors with PI3K pathway dependence. However, there were no correlation between tumor molecular alterations and clinical activity. Rodon *et al.* (71) reached the same conclusion at the final analysis of the full cohort of 83 patients and also among the subgroup of patients with breast or colorectal cancers. There were 43 patients in the expansion arm of the trial whose tumors harbored *PIK3CA* and/or PTEN mutations.

PIK3CA mutations or amplification and loss of PTEN are potential predictive markers of response because a

Table 1 Immunohistochemical studies of prognostic significance on pAkt

Studies	Sample size	Stage of disease	Types of lung cancer	PAkt antibody	Methods	Compartments IHC stained	Prognostic factor (Negative/Positive)
Yip <i>et al.</i> (103) [2014]	471	IB	NSCLC	Ser 473	IHC	Cytoplasm Nucleus	Yes (Negative)
Vincent <i>et al.</i> (104) [2011]	29	I & IIA	NSCLC	Ser 473 Thr 308	Western blotting	N/A	Not assessed
Al-Saad <i>et al.</i> (102) [2009]	335	I-III A	NSCLC	Ser 473 Thr 308	IHC	Cytoplasm Nucleus	Yes (Negative)
Tsurutani <i>et al.</i> (31) [2006]	230	I-IV	NSCLC	Ser 473 Thr 308	IHC	Not specified	Yes (Negative)
Lim <i>et al.</i> (101) [2007]	59	I, IV	NSCLC	Ser 473	IHC	Not specified	Yes (Negative)
Tang <i>et al.</i> (100) [2006]	102	I-IV	NSCLC	Ser 473	IHC	Cytoplasm Nucleus	Yes (Negative)
Shah <i>et al.</i> (105) [2005]	82	I-III A	NSCLC	Ser 473	IHC Western blotting, WB	Cytoplasm Nucleus Membranous	No in pAkt expression by IHC Yes in pAkt/ α -actin by WB (Positive)
Balsara <i>et al.</i> (29) [2004]	110	I-IV	NSCLC	Ser 473	IHC	Cytoplasm Nucleus	No
Hirami <i>et al.</i> (106) [2004]	80	Not specified	NSCLC	Ser 473	IHC	Cytoplasm	Yes (Negative)
David <i>et al.</i> (99) [2004]	61	I-IV	NSCLC	Ser 473	IHC	Cytoplasm Nucleus	Yes (Negative)
Massion <i>et al.</i> (46) [2004]	242 (NSCLC 215)	I-IV	NSCLC & limited- extensive stage SCLC	Ser 473	IHC	Cytoplasm Nucleus	No
Mukohara <i>et al.</i> (98) [2004]	91	I	NSCLC	Ser 473	IHC	Cytoplasm Nucleus	No
Mukohara <i>et al.</i> (97) [2003]	60	I-III	NSCLC	Ser 473	IHC	Cytoplasm Nucleus	No
Tsao <i>et al.</i> (96) [2003]	76	I-IV	NSCLC	Ser 473	IHC	Cytoplasm Nucleus	No
Lee <i>et al.</i> (95) [2002]	43	Node +	NSCLC	Ser 473	IHC	Cytoplasm Nucleus	No

preclinical study has demonstrated that squamous cell lung cancer cells lines with PIK3CA mutations or amplifications and loss of PTEN protein expression are more sensitive to pictilisib (42).

As for prognostic biomarkers, there have been many evaluated but none have been used in daily clinical practice. The overexpression of phosphorylated Akt (pAkt) using immunohistochemistry is a promising potential prognostic biomarker, which is expressed in both squamous and

non-squamous cell lung carcinoma (95). Earlier studies (29,46,95-98) demonstrated that pAkt has no prognostic value while later studies suggested the contrary. David *et al.* (99) was the first to demonstrate that high level of pAkt expression correlated with shortened survival and was an independent prognostic factor. Subsequently, more studies further confirmed that overexpression of pAkt (Ser 473) assessed by immunohistochemistry is a negative prognostic factor in NSCLC (31,100-103) (Table 1).

Interestingly, Shah *et al.* (105) demonstrated that expression of pAkt assessed by immunohistochemistry did not correlate with patient prognosis. However, high levels of pAkt protein assessed by Western blotting and semiquantitative densitometry correlated with a good prognosis in both univariate and multivariate analysis. This highlighted the difficulties in identifying validated prognostic markers that are applicable to clinical use because studies included patients with heterogeneous stages of disease, used different immunohistochemical protocols and pAkt antibodies against different phosphorylation sites (Ser 473 *vs.* Thr 308), while others had small sample sizes, which were underpowered to demonstrate prognostic significance.

Conclusions

PI3K-Akt-mTOR signaling pathway is the target of many novel inhibitors. In order for patients to enjoy the maximal benefits and minimize side effects of these targeted therapies, identification of predictive markers are paramount. Incorporating biomarker analysis and patient enrichment strategies in clinical trials are essential in translational lung cancer research.

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