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The PI3k inhibitors: new hopes in the battle against advanced NSCLC

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1. ABSTRACT

In terms of both incidence and mortality, lung tumor is the most common cancer in the world today. Among lung tumors, 80% are classified as non-small-cell lung cancer (NSCLC) and are mostly diagnosed at an advanced stage (either locally advanced or metastatic disease). Platinum-based doublet chemotherapy, the standard treatment for advanced NSCLC, has reached a plateau of effectiveness and achieves mostly partial responses in only 30%-40% of patients and a modest survival increase. Thus, the search for new molecularly targeted therapies is mandatory. The phosphatidylinositol 3-kinase (PI3K) pathway is frequently over activated in human cancers playing a critical role both in the initiation and progression of NSCLC. Activating mutations of this pathway play a role in the development of resistance to chemotherapy and to the Epidermal Growth Factor Receptor Tyrosine Kinase inhibitors (EGFR-TKIs) erlotinib and gefitinib. These mutations are observed in 2-5 % of non-squamous NSCLC and 8-10 % of squamous NSCLC. In this paper, we describe the available data and the possible future role of PI3k inhibitors in the treatment of advanced NSCLC.

2. INTRODUCTION

Lung cancer is the leading cause of cancer related mortality for both men and women in the United States and in Europe. In the United States, in 2012, there is estimated to be 226,160 new cases of lung cancer diagnosed and 160,340 deaths from the disease (1). It comprises two main groups: non small cell lung cancer (NSCLC) which accounts for about 85% of all lung cancers, including squamous cell carcinoma, adenocarcinoma, large cell carcinoma, and small-cell lung cancer (SCLC) for the remaining 15% (2). In the last few decades, the distribution of the histological subtypes has changed, in fact there is a growing incidence of adenocarcinoma and a concurrent decline in the incidence of squamous cell carcinoma (3) and this is probably due to the changes in cigarette designing (lower tar and nicotine) (4). Even if smoking remains the primary risk factor for the development of lung cancer, approximately 20% of cases of NSCLC occurs in never (< 100 cigarettes/life) or light smokers (< 10-15 pack-years) (5).

Many patients (approximately 70%) have advanced disease at time of diagnosis, either locally advanced or metastatic disease. Platinum-based doublet

chemotherapy is considered standard of care worldwide for patients with advanced NSCL (6). This conventional first line treatment has reached a plateau of effectiveness in improving outcomes and so new treatment approaches have been developed. In the last few years some active chemotherapeutic, anti-angiogenesis and target agents have been approved deriving only modest clinical benefits.

Therefore, interest in individualizing patient treatment to maximize clinical benefit has become a focus of clinical and scientific investigation. This "personalized therapy" has become a reality for patients with NSCLC harbouring specific gene mutations.

The phosphatidylinositol 3-kinase (PI3K) signalling pathway is frequently over activated in human cancer playing a critical role both in the initiation and progression of NSCLC. This pathway is complex; however, activating mutations of PI3K pathway play a role in the development of chemo-resistance to platinum based chemotherapy and are associated with resistance to epidermal growth factor (EGFR) tyrosine kinase inhibitors (TKIS) erlotinib and gefitinib therapy. In this paper we describe the role of the PI3K pathway inhibitors in the treatment of advanced NSCLC.

3. TREATMENT FOR PATIENTS WITH NO DRIVER MUTATION

Various meta-analyses suggest a slight superiority of platinum-based schemes with gemcitabine or docetaxel compared to other combinations, resulting in a median overall survival of about 10 months with mostly partial responses in only 30% - 40% of patients (6-12).

Recent trials involving new agents have demonstrated varying responses to therapy based on tumor histology. The combination of cisplatin and pemetrexed compared with combination of cisplatin and gemcitabine in chemotherapy-naïve patients, improved survival (median overall survival (OS) 11.8 months versus 10.4 months, hazard ratio (HR) 0.90 with confidence interval 95%, 0.79-1.02) and response rates (28.9% vs. 21.7%) in the subgroup of patients with adenocarcinoma resulting in a lower incidence of severe neutropenia, severe anemia and in a higher incidence of nausea and vomiting (13-14). Based on these results, pemetrexed in combination with cisplatin has been approved as first-line treatment of patients with advanced NSCLC other than predominantly squamous cell histology.

Moreover bevacizumab, a recombinant humanized monoclonal antibody that selectively binds to and neutralizes the biological activity of human vascular endothelial growth factor (VEGF), is currently licensed for use in combination with carboplatin plus paclitaxel or cisplatin plus gemcitabine for the first line therapy in patients with non-squamous cell histology (15). Infact, in the phase III trial (ECOG4599) randomizing chemotherapy-naïve patients with no squamous-cell tumors, no brain metastases, no clinically significant hemoptysis, the chemotherapy regimen with bevacizumab showed a statistically significant advantage in OS (12.3 versus 10.3 months; HR 0.79 CI 95% 0.67-0.92) and in objective response (35% versus 15%) (16). A retrospective analysis regarding outcomes based on cancer histology showed an increased OS for adenocarcinoma patients receiving bevacizumab plus chemotherapy compared to patients treated with chemotherapy alone (14.2 vs. 10.3 months) (17). In another phase III trial (AVAIL) bevacizumab prolonged progression free survival in patients treated with cisplatin plus gemcitabine but no difference in median OS was observed among all treatment groups (18).

In the second-line setting two agents are currently approved: docetaxel and pemetrexed as chemotherapy. Docetaxel increases overall survival (7.0 vs. 4.6 months, p=0.047) compared to supportive care producing also a benefit in some domains of quality of life (19-20). There are no survival differences between the two different schedules (weekly administration vs. every 3 weeks) but a confirmed significantly different toxicity profile. In fact the risk of febrile neutropenia is significantly lower with weekly schedule (21).

Pemetrexed compared to docetaxel showed non-inferiority efficacy in all patients (22) and a better activity in adenocarcinoma or large cell carcinoma patients than docetaxel with a longer overall survival (9.3 vs. 8.0 months, HR 0.78; p=0.047) and a little longer PFS (3.1 vs. 3.0 months, HR 0.82; p=0.076) (23). In conclusion, docetaxel can be used in all histological types, while the use of pemetrexed is currently limited to tumors with non-squamous histology.

Another strategy is the maintenance treatment consisting of administration of chemotherapeutic agent or molecularly targeted agent at the end of a defined number of initial chemotherapy cycles, after achieving tumor control in an individual patient. This approach consists of drugs included in the induction regimen (continuing maintenance therapy) or other non-cross resistant agents (switch maintenance).

Pemetrexed is licensed for use as maintenance treatment in patients whose disease has not progressed after four cycles of platinum based chemotherapy with different agents. Infact, when pemetrexed was compared to placebo in this setting of patients the results were very interesting: OS was 13.4 months with pemetrexed and 10.6 months with placebo (HR 0.79; 95% CI, 0.65-0.95; p=0.012). The significant advantage in OS favouring pemetrexed becomes stronger in patients with non-squamous histotype (15.5 vs. 10.3 months in the pemetrexed and the placebo arms respectively; HR 0.70; 95% CI, 0.56-0.88; P=0.002) (24). In another phase III trial, PARAMOUNT, randomizing patients with advanced non-squamous NSCLC who had not progressed on four cycles of cisplatin plus pemetrexed induction chemotherapy, pemetrexed resulted in a statistically significant 22% reduction in risk of death (HR 0.78; 95% CI,0.64-0.96); the OS rate at 24 months was 58 and 45 months for pemetrexed and placebo arm respectively while the median OS, measured from

randomization, was 13.9 months on the pemetrexed arm and 11,0 months on the placebo arm. This study met its primary endpoint showing that pemetrexed continuation maintenance is an effective and well tolerated treatment for patients with advanced non-squamous NSCLC (25, 26).

Target agent Erlotinib, an EGFR TKIs, was approved for treatment of unselected patients as second or third line therapy. In this setting, erlotinib improves not only RR, PFS and survival (advantage of 2 months compared to placebo) (27), but also quality of life reducing the mayor symptoms related to the disease as cough, dyspnoea and pain. Moreover, smoking status appeared to be the most important predictor of a survival benefit with erlotinib (28).

Also erlotinib was evaluated as switch maintenance. In the SATURN randomized phase III trial, patients with no evidence of disease progression after four cycles of platinum-based chemotherapy were randomized to receive erlotinib or placebo until progression or unacceptable toxicity. In all patients erlotinib showed a survival advantage that was superior both in patients with non-squamous histology and patients with EGFR wild-type and it was higher in patients achieving stable disease after chemotherapy than in those achieving partial or complete response (29, 30). So, EMEA approved erlotinib as maintenance treatment after platinum-based chemotherapy in advanced NSCLC patients achieving stable disease after chemotherapy while FDA approved erlotinib for all patients whose disease has not progressed after four cycles of first-line platinum based chemotherapy.

4. TREATMENT FOR PATIENT WITH DRIVER MUTATION

In 2004, the identification of somatic mutations in the EGFR gene provided the first glimpse of a clinically relevant NSCLC oncogene. The most common mutations are an in frame deletion in exon 19 around codons 746 to 750 (45-50% of all somatic EGFR mutations) and a missense mutation in exon 21 (35-45% of all EGFR mutation) (31,32), these mutations, within the kinase domain of EGFR, activate the EGFR-signalling pathway in the absence of ligand, and promote EGFR-mediated pro-survival and anti-apoptotic signals through downstream of other pathways such as phosphatidylinositolo-3-kinase (PI3K)/protein kinase B (AKT). Activating mutations, in the EGFR gene, are related to the response to EGFR TKI showing a higher OS and PFS in mutated patients treated with an EGFR-TKI and are more frequently detected in a subpopulation of NSCLC patients: female sex, non smokers, Asian origin, adenocarcinoma histology (33-36). Two EGFR-TKIs have reached regulatory approval for the treatment of NSCLC: erlotinib and gefitinib, two small molecules that are administered orally, daily and inhibit the tyrosine kinase activity of EGFR.

Four randomized phase III trials evaluated the role of gefitinib as first line therapy. In the IPASS and in the First-Signal trials, patients were selected according to clinical factors (never or former light smoker, adenocarcinoma, Asian ethnicity) to receive gefitinib or chemotherapy. In both trials, the median PFS at 1 year was superior in the gefitinib compared to chemotherapy group with no difference in OS between the two treatments. Farther, a subgroup analysis showed a longer PFS and a higher RR in EGFR mutations positive patients treated with gefitinib than in those treated with chemotherapy. The gefitinib administration was associated with a clinically relevant improvement in quality of life and with a lower rate of severe adverse events, where the most common adverse events were rash or acne (37, 38). In the other two trials only patients harbouring EGFR mutations were randomized to receive gefitinib or chemotherapy. In these two studies, PFS and RR were higher in patients treated with gefitinib (39, 40). Based on these results the EMEA approved gefitinib for the treatment of advanced NSCLC patients harbouring EGFR mutations even in first line setting.

About erlotinib, two randomized phase III trials were performed to evaluate its action in advanced NSCLC patients harbouring EGFR mutations: the OPTIMAL trial conducted in Asian patients and the EURTAC trial developed in Europe. In these trials the PFS and RR were significantly longer in erlotinib arm than chemotherapy group (41, 42). On these results EMEA approved erlotinib for the treatment of chemo-naïve patients with advanced NSCLC harbouring EGFR mutations.

Afatinib, a next generation TKI that irreversibly inhibits human epidermal growth factor receptor 2 (Her2) and EGFR kinases, has been submitted to the FDA, EMA for treatment of EGFR mutation positive locally advanced and metastatic NSCLC patients. In patients harbouring EGFR mutation, treatment with afatinib significantly prolonged PFS compared to chemotherapy. In addition patient treated with afatinib experienced better and longer control and improvement of the most common lung cancer related symptoms and better quality of life compared to chemotherapy (43-44) (Table 1).

Subsequently other genetic abnormalities have been identified such as, echinoderm microtubule-associated protein like 4 (EML4) and anaplastic lymphoma kinase (ALK) gene fusion. The EML4-ALK protein results in constitutive dimerization of the kinase domain of ALK leading to aberrant activation of downstream signaling such as Akt, STAT3, and extracellular signal regulated kinase 1 and 2 (ERK1/2) [45-46). This rearrangement occurs in around 5 % of lung cancer quite exclusively in lung adenocarcinoma, young age, never- or former-smoker and usually independently of EGFR and KRAS gene mutations. Crizotinib, the first-in-class dual ALK and c-MET inhibitor, is approved in Europe for the treatment of adults with previously treated ALK-positive advanced NSCLC. Infact, it has been particularly effective against ALK-rearranged NSCLC (47) showing dramatic and prolonged responses : in the PROFILE 1007, a phase III randomized trial, crizotinib doubled PFS to a median 7.7 months compared with 3 months for chemotherapy (HR 0.49) and trebled the RR of chemotherapy (65% vs. 20%, $p< 0.0001$). (48)

Table 1. Main target therapy studies in patient with EGFR activating mutations

Target agent	Trials	Regimen treatment	Progression Free Survival/ Hazard Ratio	Response rate
Gefitinib	IPASS (37)	Gefitinib Vs CBDCA + PTX	9.5 months/ HR 0.48 Vs 6.3 months	71,2% Vs 47,3%
Gefitinib	FIRST-SIGNAL (38)	Gefitinib Vs CDDP + GEM	8.4 months / HR 0.61 Vs 2.1 months	84.6% Vs 25.9%
Gefitinib	WJTOG3405 (39)	Gefitinib Vs CDDP + TXT	9.2 months / HR 0.489 Vs 6.3 months	62,1% Vs 32,2%
Gefitinib	NEJ002 (40)	Gefitinib Vs CBDCA + PTX	10.8 months/ HR 0.36 Vs 5.4 months	73,3% Vs 30,7%
Erlotinib	OPTIMAL (41)	Erlotinib Vs CBDCA + GEM	13.1 months/ HR O.16 Vs 4.6 months	83% Vs 36%
Erlotinib	EURTAC (42)	Erlotinib Vs PBC	9.7 months/ HR 0.37 Vs 5.2 months	64% Vs 18%
Afatinib	LUX-LUNG 3	Afatinib Vs CDDP+PEM	11.1 months/ HR 0.58 Vs 6.9 months	56% Vs 23%
Afatinib	LUX-LUNG 6	Afatinib Vs CDDP+GEM	11.1 months / HR 0.28 Vs 5.6 months	66 ,9% Vs 23%

CBDCA: carboplatin; PTX: paclitaxel; CDDP: cisplatin; GEM: gemcitabine; TXT: docetaxel; PBC: platinum-based chemotherapy; PEM: pemetrexed.

5. PHOSPHATIDYL INOSITOL 3-KINASES (PI3Ks) PATHWAY

The phosphatidylinositol 3-kinases (PI3Ks) are a family of lipid kinases divided into three classes (class I, II and III) based on the substrate, structure, mechanism of activation and function (49). Among these classes, class I PI3Ks are divided in two subgroups, class IA and class IB where the class IA is the one most clearly implicated in cell proliferation, growth and survival while class IB appears involved in immune function and inflammation (50). Class II is involved in the regulation of membrane trafficking whereas class III is implicated in the regulation of autophagy (49). Class IA consists of a regulatory subunit p85 and a catalytic subunit p110; three genes, PIK3R1, PIK3R2, PIK3R3, encode five p85 isoforms as a consequence of splice variants and each p85 isoform can associate with any of the three p110 isoforms (p110α, p110β,p110δ) encoded by three different genes, PIK3CA, PIK3CB and PIK3CD, respectively. Both PIK3CA and PIK3R1 are somatically mutated in cancers promoting activation of the PI3K pathway (51).

Class IA PI3Ks are activated by growth factor stimulation through receptor tyrosine kinases (RTKs), in fact when a growth factor binds its RTK (including the human epidermal growth factor receptor family, platelet-derived growth factor receptor and the insuline-like growth factor receptors) this causes the RTK dimerization and autophosphorylation binding the p85 subunit. This binding removes the inhibitory effect of p85 on p110 and localizes PI3K to the plasma membrane where its substrate, PIP2 (phosphatidylinositol-3, 4, 5-diphosphate), resides. The activated kinase, phosphorylates PIP2 on the 3'OH position to produce PIP3 (phosphatidylinositolo-3, 4, 5-triphosphate) resulting in activation of second messenger molecules that regulate a variety of physiological cellular metabolic and survival functions (50, 52, 53).

PI3K can also be activated by RAS which directly interacts with the p110 subunit (54); additionally this subunit can be activated by G-protein coupled receptors. PIP3 facilitates the phosphorylation of Akt (a serine-threonine protein kinase) at threonine-308 in its kinase domain by phosphoinositide dependent kinase 1 (PDK1); a second phosphorylation event at serine 473 in the helical domain of Akt by mTOR complex is necessary for full Akt activity (55).

Akt is the central mediator of the PI3K pathway, in fact it promotes cell survival by inactivating cell cycle inhibitors such as p27 and p21 and by inhibiting pro-apoptotic genes (Fas Ligand,BAX,BAD); moreover phosphorilation of Mdm2 by Akt antagonizes p-53 mediated apoptosis reducing programmed cell death. Also, Akt stimulates protein synthesis and cell growth by activating mTOR trough the tuberous sclerosis complex (TSC) so mTOR complex 1 relays signals following PI3K-AKT activation while mTOR complex 2 contributes to complete Akt activation (52,56,57).

PI3K regulates cellular metabolism by enhancing glucose uptake and glycogen synthase activity in muscle and fat and by inhibiting gluconeogenesis in the liver (58).

The PI3K pathway activity is down regulated by phosphate and tensin homologue deleted on chromosome 10 (PTEN). PTEN, originally identified as a tumor suppressor gene located on chromosome 10q23, antagonizes PI3K signalling by dephosphorylating the second messenger PIP3 forming the inactive PIP2; it also acts in the nucleus to further impact cell cycle, apoptosis and chromosomal integrity (59). (Figure 1)

Extensive crosstalk exists with other cellular signalling networks for example activation of the tumor suppressor p53 causes both increased PTEN and decreased p110 expression with PI3K reduced activity, however PTEN reduces indirectly p53 degradation inhibiting PI3K activation (60).

PI3K pathway is activated in human cancers by several different mechanisms. Increased PI3K signalling is often due to genetic mutations and aberrations affecting various components of pathway and the consequences of each alteration depend on which pathway factor is affected and whether other tumor genes are concomitantly deregulated (61,62).

The most common PI3K pathway alteration founds in human cancer is inactivation of PTEN resulting in aberrant PI3K pathway activation; in fact both mutations and decreasing levels of PTEN expression are correlated with the progressive outcome of solid cancers (63). More recently, somatic mutations in PIK3CA, the gene encoding p110α, have

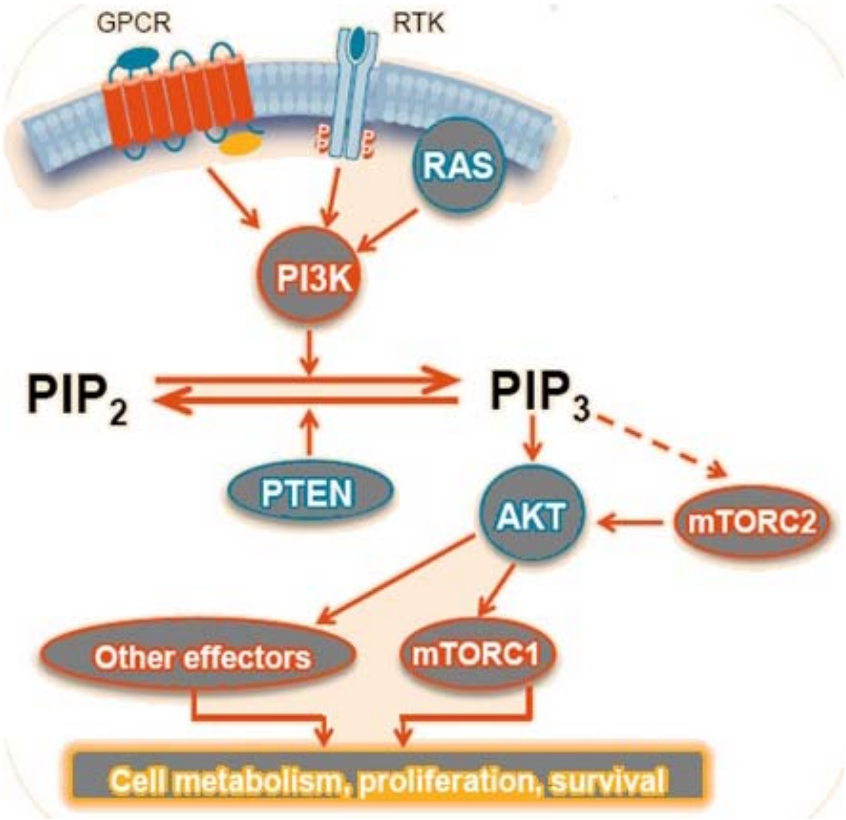

Figure 1. The phosphatidylinositol 3 kinase (PI3K) is activated by growth factor stimulation through receptor tyrosine kinase (RTKs), RAS and by G-protein coupled receptors (Gpcr). PI3K signalling is negatively regulated by PTEN (phosphate and tensin homologue deleted from chromosome 10). The activated kinase, phosphorylates PIP2 on the 3'OH position to produce PIP3 (phosphatidylinositolo-3,4,5-triphosphate) resulting in activation of second messenger molecules that regulate a variety of physiological cellular metabolic and survival functions Akt activates downstream effectors, including mTORC1 (mammalian target of rapamycin complex 1). mTOR complex 1 relays signals following PI3K-AKT activation while mTOR complex 2 contributes to complete Akt activation. PI3K plays a key role in multiple cellular processes.

been identified in a variety of human tumors. These mutations affect two hot spot regions in exon 9 and 20 encoding the helical domain and the catalytic domain of p110α, respectively. The catalytic domain mutations may constitutively activate its enzymatic activity while the helical domain mutations may affect interaction with regulatory proteins as p85. These mutations cause constitutive activation along the PI3K pathway in the absence of growth factor signal obviating the usual obligate interactions with phosphorylated RTKs (64, 65); thus the presence of these mutations confers resistance to therapies targeting RTKs (66). Also, mutations or amplification in AKT family genes have been identified in human cancers (67-69).

In NSCLC, PI3K/AKT/mTOR signalling is frequently deregulated due to mutations affecting one of its upstream regulators as showed by some studies using engineered mouse models; in fact in these studies transgenic mice with a specific p110α mutant develop lung adenocarcinomas (70,71).

PI3K pathway is not only responsible for balancing cell survival and apoptosis but plays a role in the development of chemo-resistance to platinum-based chemotherapy and to TKIs therapy. In fact, genetic variations in the genes encoding PI3K components, PTEN, AKT and mTOR can to induce variation in the development of toxicity or clinical outcomes during platinum-based therapy. Pu *et al* enrolled 168 non – Hispanic Caucasian patients with advanced stage NSCLC treated with primary platinum-based chemotherapy, from 1995 to 2004. Genomic DNA was extracted from peripheral blood lymphocytes and selected for five genes: AKT1, AKT2, PIK3CA, PTEN, FRAP1 (mTOR). These authors showed an association between some specific genetic alterations and risk of toxicity to platinum based-chemotherapy: genetic PTEN variation rs2299939 was associated with a 54% decreased risk of toxicity (HR: 0.44, 95% CI: 0.20-0.95, p=0.036) while patients with homozygous PIK3CA variant rs2699887 exhibited a significantly increased risk of toxicity. Moreover, three genetic variation in AKT1 (rs3803304, rs2498804, rs1130214) were associated with a prolonged progression-free time. Authors concluded that decreasing expression of PTEN is associated with a decrease in toxicity and that decreased PI3K activity increased apoptosis in sensitive non cancer cells causing a rise in toxic side –effects. Farther, the genetic variants in AKT1 were sufficient to

Table 2. PI3K pathway alteration in NSCLC

	PIK3CA		PTEN
Histotype	**Mutation**	**Copy number gain**	**Loss-of-function**
Squamous	8-10%	33%	15-20%
Non squamous	2-5%	6%	15-20%

d PIK3CA: gene encoding p110α subunit of phosphatidylinositol-3-kinases; PTEN: tensin homologue deleted on chromosome10.

ecrease risk for disease progression confirming the central AKT1 role in survival and in cisplatin resistance (72).

With regard to EGFR mutated tumors, it has been demonstrated that EGFR-signalling results in the activation of the PI3K/Akt pathway and activation of this signalling pathway is associated with a poor prognosis. Therefore loss of PTEN and activation of PI3K pathway can also cause resistance to erlotinib and gefitinib representing a therapeutic target for PI3K inhibitors (73).

Generally PIK3CA mutations and PTEN alterations are mutually exclusive in NSCLC and occur in 15-20% of the patients. The most frequent molecular changes are: gain-of-function mutations of oncogenes encoding for components of PI3K itself (PIK3CA) and loss-of-function mutation affecting negative regulators of PI3K as PTEN (i.e., loss of expression or function). Activating mutations of PIK3CA are observed in 2-5% of non squamous NSCLC and 8-10% of squamous NSCLC; PIK3CA copy number gain has been reported in 33% of squamous and in 6% non-squamous cell lung cancer in one large series; other molecular changes detected in NSCLC as PTEN loss and AKT1 or AKT2 over-expression have been reported in 39%, 18% and 22%, respectively (74, 75) (Table 2).

6. PI3K PATHWAY INHIBITORS

The PI3K inhibitors can be divided into two groups: isoform specific inhibitors and pan-PI3K inhibitors whereas these targ*et al*l class IA PI3K.

The most extensively studied agents are Wortmannin isolated from Penicillim Wortmanni in 1957 and LY291002 produced about 25 years ago. These agents are characterized by the ability to block all classes of PI3Ks and their administration results in potent antitumor and antiangiogenic activity across a broad spectrum of cancer cell lines especially when there is an excess in PI3K activity (76). Though, these agents have not progressed to clinical trials in fact Wortmannin showed poor stability and considerably toxicity in animal models at low doses and LY291002 showed poor pharmacokinetics, poor water solubility, and undesirable toxicity (77, 78). So novel compounds have been developed to improve pharmacokinetic profiles and achieve superior target specificity.

6.1. SF1126

SF1126 (*Semafore Pharmaceuticals*) is composed of the pan PI3k inhibitor LY291002 conjugated to an RGD targeting peptide and designed to increase solubility and binding to integrins expressed on tumor vasculature. In xenograft models, this agent is more active in tumors with an activated PI3K-Akt signalling pathway showing a significant antitumor effect and it has showed antiangiogenic activity blocking the HIF-1α vascular endothelial growth factor signalling in the tumor cell (79). Moreover, in a phase I trial enrolled patients with solid tumors, SF1126 administered intravenously in cycles of 4 weeks showed disease stabilization in multiple patients with refractory tumors and PI3K inhibition (80).

6.2. NVP-BEZ235

NVP-BEZ235 (*Novartis Pharmaceuticals*) belongs to the class of imidazoquinolines that potently and reversibly inhibits class I PI3K catalytic activity in an ATP-competitive manner. It also inhibits mTOR catalytic activity but does not target other protein kinases (81). This dual inhibition is important because mTOR inhibitors interrupt negative feedback loops that down-regulate PI3K signaling, causing paradoxical up regulation of pro-survival signalling pathways. In preclinical studies NVP-BEZ235 showed anti-proliferative and apoptotic activity against a wide range of cancer cell lines, including NSCLC cell lines. Also, Zito *et al.* showed an interesting synergism between NVP-BEZ235 and erlotinib, in fact in all NSCLC cell lines treated with these agents, erlotinib potentiated the growth inhibition with a lower dose than that used in actual patients. Moreover, this agent strongly inhibited activation of downstream effectors (e.g. Akt) and showed antitumor activity in PI3K mutated xenograft models of human cancers (82, 70, and 83). Based on these results, a phase I study was conducted in patients with histological confirmed and unresectable solid tumors. In this study, NVP-BEZ235 was administered once daily until unacceptable toxicity or disease progression. Five patients with non-small lung cancer were included in this study with two of them demonstrating response by CT and PET criteria. The treatment was well tolerated with mild or moderate adverse events such as nausea, vomiting, diarrhea, fatigue, anaemia and anorexia. Data showed that NVP-BEZ235 was more active in patients with PI3K pathway not regulated tumors (84).

6.3. XL765

XL765 (*Sanofi*) is a dual inhibitor of class I PI3K isoforms and of mTOR. Tumor stabilization or shrinkage has been observed in a variety of mouse xenograft models of human cancer including breast, ovary, lung, prostate and brain cancers. Two phase I studies were conducted to evaluate the safety and the pharmacokinetics of this agent. In the first study, authors enrolled 34 patients to receive XL765, 26 patients received a bid regimen (15- 120 mg) and 8 patients a qd regimen (70-100 mg) for 28-day cycles. Tumor response was assessed every 8 weeks by RECIST criteria. Five patients had durable stable disease; among these the patient with NSCLC receiving a dose of 30 mg bid had stable disease for 4 cycles. The most common related adverse events were nausea, diarrhea and elevated liver enzymes. PI3k inhibition signalling pathway of about

60-90% was seen in hair and skin at multiple tolerable doses (85). In the second study, XL765 was administered according two schedules: 36 patients received XL765 on days 1-21 across 7 dose levels (30-900 mg) and 3 patients received agent as a continuous daily dose at 100 mg. Tumor response was assessed every 8 weeks. The treatment was generally well tolerated with a maximum tolerated dose of 600 mg for the schedule days 1-21, the most common drug-related toxicity was skin rash. A prolonged stable disease over 10 months has been observed in 3 patients with NSCLC (86). In another phase I study, XL765 was evaluated in combination with erlotinib in patients with advanced solid tumors. The combination was generally well tolerated with no apparent PK interaction and resulted in robust inhibition of PI3K and EGFR signalling in skin and tumor tissue. Four NSCLC patients (3 had progressed on erlotinib) had been on study for 16 to 45 weeks (87).

6.4. XL147

XL147 (*Sanofi*) is a selective inhibitor of class I PI3K isoforms showing tumor stabilization in a variety of mouse xenograft models in human cancer. Moreover, enhanced antitumor effects have been demonstrated when this agent was combined with cytotoxic chemotherapy. In a phase I dose escalation study, 68 patients with solid tumors were enrolled to receive once daily XL147 on days 1-21 on /7 day off or as a continuous daily dose in 28 –day cycles. The treatment was generally well tolerated, drug-related skin rash was reported in 12% of patients including 4% of patients with grade 3 events; XL147 related SAEs included grade 4 arterial thrombosis. One patient with NSCLC had confirmed partial response (PR) with a 33% reduction in the target lesion. Inhibition of PI3K pathway signalling and phopshorylation of MEK and ERK has been demonstrated in solid tumors (88). Farther, this agent was combined with erlotinib and cytotossic chemotherapy in two phase I studies. The combination of XL147 and erlotinib was generally well tolerated with no major Pk interaction, the most frequently reported adverse events were rash (45%), nausea (30%), diarrhea, fatigue and vomiting, one patients died due to progressive disease and to drug rash with eosinophilia and systemic symptoms (DRESS syndrome). In this study, 8 NSCLC patients were enrolled but only one EGFR inhibitor-naïve had confirmed PR with 59% reduction in the sum of target lesions (89).

6.5.PI-103, GDC-0941

PI-103 (*Pfizer)* and GDC-0941 (*Genentech)* are two selective and potent inhibitors of class I PI3Ks with weaker activity against mTOR. Both agents showed a clear antitumor activity in two gefitinib-resistant non-small cell lung cancer cell lines, A549 and H460 whereas this activity was higher in H460 cell line with activating mutations of PIK3CA than in A549 cells with wild-type PIK3CA. PI-103 inhibited the cells growth by causing down-regulation of cycline D1 and E1 and up-regulation of p21 and p27 associated with arrest in the G0-G1 phase of the cell cycle. Furthermore, GDC-0941 was highly efficacious in combination with MEK inhibitor UO126 in inducing cell growth inhibition (90, 91). In a phase Ib study the clinical combination of the MEK inhibitor GDC-0973 and GDC-0941showed improved efficacy compared to either alone with toxicities similar to those observed in single agent GDC-0973 and GDC-0941 phase I trial. The most common adverse events were diarrhea (90% all G1/G2), fatigue (61%), nausea (61%), rash (50%), vomiting 33%, increased lipase (1 patient grade3) and CPK elevation (1 patient grade 4). Two patients with NSCLC had a partial response with decreased measurable target lesion (92). Also, GDC-0941 was combined with paclitaxel and carboplatin with or without bevacizumab in patients with advanced NSCLC. In the phase Ib study there were two treatment arms, in arm A patients received GDC-0941 with chemotherapy every 3 weeks while in arm B patients received bevacizumab in addition to chemotherapy and GDC-0941. In both arms, the PI3K inhibitor was given once daily on days 1-14 of a 21-day cycle. The treatment was well tolerated, treatment related adverse events occurred in 20 % of patients and all were Grade 1 and 2 including alopecia, asthenia, nausea, stomatitis, arthralgia, peripheral neuropathy and rash. In 8 of 18 patients enrolled, confirmed partial responses were seen with a median follow-up of 5.4 months at the time of data cut-off (93).

6.6.PX-866

PX-866 (*Oncothyreon)* is another pan-class I PI3K inhibitor similar to Wortmannin although it acts in an irreversible manner. Studies *in vivo* utilizing xenograft models derived from cell lines of various tissue origins showed that mutant PIK3CA and loss of PTEN activity without mutant Ras were sensitive to PX-866 antitumor activity while mutant oncogenic Ras was a negative predictor of response to this inhibitor even in xenografts with concurrent activating mutation in PIK3CA, thus this mutation cannot be used as individual marker for sensitivity (94). Also, PX-866 evaluated in gefitinib-resistance NSCLC cell line A-549 potentiated the antitumor activity of the EGFR inhibitor gefitinib against even large A-549 NSCLC xenografts with complete tumor growth control in the early stages of treatment inhibiting tumor Akt phosphorylation which was not seen with gefitinib alone. Prolonged PX-866 administration caused increased neutrophil counts and hyperglycemia with decreased glucose tolerance which was insensitive to metformin but reversed by insuline (95). In a phase I dose escalation study, patients with advanced solid tumors received escalating doses of PX-866 orally once daily on days 1-5 and 8-12 of a 28 day cycle with an expanded cohort at the maximum tolerated dose (MTD). Evaluation of daily continuous schedule began once MTD was determined for intermittent schedule. Authors treated 52 patients and 7/31 evaluable patients had stable disease receiving > 4 cycles. Most adverse events were nausea, vomiting, diarrhea, anorexia and hypertension. No changes in glucose levels were seen (96).

6.7.BKM120

BKM120 (*Novartis)* is a potent and highly specific oral pan-class I PI3K inhibitor, currently tested in clinical trials. This agent demonstrated significant tumor growth inhibition in relevant tumor xenografts in mice and rats when administered orally, including models of breast cancer, lung cancer, colon cancer. Some preclinical experiments have been conducted, approximately 50% of

Table 3. Inhibitors against PI3K pathway

Agent	Molecular targets	Monotherapy/Combination	Phase study
NVP-BEZ235 (Novartis)	PI3K/mTOR	Monotherapy	Phase I
		combined with ERLOTINIB	
XL765 (Sanofi)	PI3K/mTOR	Monotherapy	Phase I
		combined with ERLOTINIB	
XL147 (Sanofi)	PI3K	Monotherapy	Phase I
		combined with ERLOTINIB	
PI-103 (Pfizer)	PI3K/mTOR	Monotherapy	Phase I
GDC-0941 (Genentech)	PI3K/mTOR	Monotherapy	Phase I
		combined with MEK inhibitor	
PX-866 (Oncothyreon)	PI3K	Monotherapy	Phase I
BKM120 (Novartis)	PI3K	Monotherapy	Phase I / phase II
		combined with ERLOTINIB	

PI3K: phosphatdylinositol-3kinases; mTOR: mammalian target of rapamycin; NSCLC: non small cell lung cancer. Some phase I trials are testing both the single agent and the combination with Erlotinib. In fact in NSCLC cell lines treated with this combination, erlotinib potentiated the growth inhibition.

the tested NSCLC cell lines showed sensitivity to BKM120 (15 out of 28 non squamous cell lines; 3 out of 7 squamous cell lines); given the low rate of PIK3CA and PTEN mutations in NSCLC, only two lines carrying these mutations have been tested. However, KRAS mutations showed no impact on the sensitivity to this inhibitor *in vitro*. Preliminary clinical data with BKM120 in patients with advanced NSCLC has been obtained in the phase I study enrolling three patients with NSCLC with two stable diseases. In this study 77 patients were enrolled, partial tumor responses were observed in two patients harbouring PIK3CA mutation while stable diseases were observed in 26 of 45 evaluable patients. Most frequent adverse events of all grades were decreased appetite (33%), rash, diarrhea, nausea, fatigue, anxiety (20%), depression (18%) and mucositis (17%) (97) (Table 3). A prospective multi-center, open label, phase II study to investigate the efficacy and safety of BKM120 in adult patients with metastatic NSCLC and activated PI3K pathway activation is ongoing (Clinical Trial.gov Identifier: NCT01297491) (98). In this trial, BKM120 will be investigated in two groups of patients according to the histology: squamous and non squamous.

7. CONCLUSION

In patients with metastatic squamous NSCLC that progressed after platinum-based first-line therapy, chemotherapy or erlotinib represent the options for second-line treatment (99, 39). In the subgroup of patients with non-squamous NSCLC without EGFR mutation or unknown EGFR status, after the failure of first-line chemotherapy for metastatic disease, the available treatment options are chemotherapy with pemetrexed (if not used before) and Docetaxel or erlotinib, all considered unsatisfactory. In tumors with activating EGFR mutations, most patients have an initial clinical response of 60 to 80% when treated with EGFR TKIs. However, the majority of these patients inevitably develop acquired resistance to these agents after a median approximately 10 months from the initiation of treatment. In nearly half of these cases, resistance is caused by replacement of threonine by methionine at position 790 (T790M) in exon 20 of the EGFR Kinase domain increasing the ATP binding affinity of EGFR approximately 10 fold in the presence or absence of a TKI and allows ATP to competitively displace gefitinib and erlotinib from EGFR (100). In fact, neither gefitinib nor erlotinib can circumvent this resistance, as demonstrated by the lack of activity with erlotinib in patients that acquired resistance to gefitinib. But in the other cases a second mechanism of resistance to EGFR TKIs is the activation of parallel pathways so that tumor cells may become independent of EGFR signalling such as the MET amplification or activation of PI3K by other receptor tyrosine kinases or by genetic mutations and aberrations affecting various components of pathway. The optimal approach to treatment of such patients remains undefined. There is good reason to believe that the resistance observed in such patients is only partial, in fact it is hypothesized that in patients with acquired resistance to Erlotinib treatment with PI3k inhibitor continuing Erlotinib can restore the cell sensitivity to TKI. Furthermore, preclinical data showed that lung cancer cell lines harbouring EGFR mutation and acquired resistance to Erlotinib were more sensitive to BKM120 activity. So, a trial combining BKM120 with Erlotinib in patients with acquired resistance to Erlotinib (evidence of disease control with erlotinib for at least 6 months) is ongoing (Clinical trials.gov Identifier: NCT01487365) (101).

The role of PI3ks pathway in advanced NSCLC and the evidence that activating mutations of PI3K pathway play a role in the development of chemo-resistance to platinum based chemotherapy and resistance to erlotinib and gefitinib therapy, open the door to new treatment options in the battle against advanced NSCLC.

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