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Lung cancer therapeutics that target signaling pathways: an update

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Abstract

Claiming more than 150,000 lives each year, lung cancer is the deadliest cancer in the USA. First-line treatments in lung cancer include surgical resection and chemotherapy, the latter of which offers only modest survival benefits at the expense of often severe and debilitating side effects. Recent advances in elucidating the molecular biology of lung carcinogenesis have elucidated novel drug targets, and treatments are rapidly evolving into specialized agents that hone in on specific aspects of the disease. Of particular interest is blocking tumor growth by targeting the physiological processes surrounding angiogenesis, pro-tumorigenic growth factor activation, anti-apoptotic cascades and other cancer-promoting signal transduction events. This article looks at several areas of interest to lung cancer therapeutics and considers the current state of affairs surrounding the development of these therapies.

Keywords

angiogenesis; apoptosis; cancer stem cells; lung cancer; monoclonal antibody; signal transduction; targeted therapy; TKI; tyrosine kinase inhibitor

Lung cancer is the number one cancer killer in the world for both men and women. In the USA, lung cancer claims more than 150,000 lives annually – more than breast, colon and prostate cancers combined [1]. Over 200,000 Americans will be diagnosed with the disease in 2010, of whom only 15% are expected to survive beyond 5 years [1]. This dismal 5-year survival statistic has remained virtually unchanged since the 1970s [2]. Non-small-cell lung cancers (NSCLCs) – including adenocarcinoma (and its bronchoalveolar carcinoma [BAC] subset), squamous cell carcinoma, large cell carcinoma and undifferentiated NSCLC – account for 80% of lung cancers [2].

Complete surgical resection, which offers a 40% 5-year survival rate, remains the best option for treatment, but over 75% of patients present with advanced or metastatic disease at the time of diagnosis and are unsuitable for surgery [3]. Platinum-based chemotherapy regimens are the standard therapy for NSCLC, but such regimens are associated with severe toxicities, and efficacy of the treatment is limited due to multiple drug resistance of NSCLC cells [4].

In recent years, it has become increasingly clear that a therapeutic plateau has been reached for treating advanced-stage NSCLC patients with conventional chemotherapeutics [5]. Improved understanding of tumor biology and the molecular basis of carcinogenesis has heralded the advent of targeted agents as therapeutics for treating lung cancer (Table 1). Research efforts are focused on developing novel agents that target apoptosis, angiogenesis and tumor growth pathways in particular (Figure 1).

Targeting EGFR

The EGF receptor (EGFR) belongs to the ErbB receptor tyrosine kinase family and is the key receptor for the EGF family [6]. Aberrant activation of EGFR has a known association with five out of the six hallmarks of cancer: by passing apoptotic cell death, self-sufficient growth, lack of response to anti-growth signals, sustained angiogenesis and tumor metastasis [6-8]. Although EGFR itself is the most studied of the ErbB receptor kinases, deregulation can occur at a number of points along the pathway [8]. Deregulation of EGFR has been implicated in 30% of all epithelial tumors, and presents in a number of manners, including enhanced ligand production by cancerous cells, increased EGFR expression on cancer cell membranes, and activating mutations of *EGFR* and related genes [9]. Over 80% of NSCLC patients, have detectable levels of EGFR protein in their tumors according to the National Comprehensive Cancer Network [301].

Owing to the prevalence of EGFR overexpression in NSCLC tumors, EGFR has been under investigation for some time as a target for therapeutics. In addition to monoclonal antibodies (mAbs) that target its extracellular binding site, much focus has been on discovering small molecules that bind the EGFR tyrosine kinase. Erlotinib and gefitinib were the first generation of tyrosine kinase inhibitors (TKIs) approved for the treatment of NSCLC [10]. Patient response to erlotinib is strongly correlated with the patients' tumors containing somatic activating mutations in the *EGFR* gene, generally between exon 18 and 21 (and most commonly exon 19 deletion and the L858R mutations) [11]. The overall NSCLC patient population shows a 10–20% response to these treatments, and both erlotinib and gefitinib are approved as second- or third-line treatment agents [10]. Although less than a third of NSCLC patients respond to EGFR TKIs, more than 90% of those who do harbor EGFR mutations [12,13]. A large Phase III trial in Asian patients with gefitinib as a first-line treatment compared with standard chemotherapy showed a dramatic progression-free survival benefit in patients receiving gefitinib alone, but overall survival remained essentially unaltered [14]. Because response to EGFR inhibitors is associated with the presence of sensitizing mutations in the gene, several standard methods such as direct sequencing, PCR, fluorescent *in situ* hybridization and immunohistochemistry (IHC) are currently used to detect EGFR amplification and mutations to predict whether patients are likely to benefit from EGFR-TKI therapy [15]. Unfortunately, despite the remarkable clinical progress made by patients responsive to these drugs, prolonged administration results in acquired resistance by several mechanisms including a secondary mutation in the *EGFR* gene and amplification of the mesenchymal-epithelial transition (*c-MET*) proto-oncogene [16].

Amplification of *c-MET* has a known association with both TKI resistance and poorer NSCLC prognosis [17]. A Phase II trial investigating ARQ197, an orally administered small-molecule inhibitor of *c-MET*, demonstrated that the drug, when administered in conjunction with erlotinib, significantly improved progression-free survival compared with use of erlotinib alone, but no effect on overall survival was noted. However, the drug appeared to particularly benefit patients with *KRAS* mutations, nonsquamous histologies and wild-type EGFR status [18].

Efforts are now underway to develop a new generation of EGFR TKIs that respond to secondary resistance. HKI-272 (neratinib) targets two receptors in the ErbB family, EGFR and human EGF receptor (HER)2, and has completed Phase II trials in both untreated patients and those with acquired resistance to first-generation TKIs. Although the results of the trial were generally disappointing – neratinib showed little activity overall – patients with the rare 18G719X point mutation experienced a notable response. Three of the four patients with the point mutation responded partially, and the fourth had stable disease for more than 40 weeks. Strikingly, two of these patients (including the one with stable disease) had transitioned to neratinib directly from erlotinib, validating the former compound's efficacy in combating TKI-refractory disease [19]. XL647 is an inhibitor of EGFR, HER2 and VEGF receptor (VEGFR), and has been shown to inhibit the growth of cell lines refractory to first-generation TKIs [20]. BIBW2992 also inhibits EGFR and HER2 and has been shown to inhibit wild-type EGFR, EGFR exon 19 deletion and EGFR L858R (all acquired resistance mutations) *in vitro*. The drug's efficacy is, however, diminished by a secondary T790M mutation conferring TKI resistance, but new *in vitro* and *in vivo* assays demonstrate synergy between BIBW2992 and other chemo therapeutics, such as thymidylate synthase-targeting drugs [21]. Both BIBW2992 and XL647 have promising preliminary findings from Phase I and II trials [20]. PF299804, a potent irreversible inhibitor of EGFR, HER2 and HER4, also shows promise as a second-generation TKI for use in erlotinib-refractory patients. A Phase II trial investigating the activity of PF299804 compared with erlotinib found that the former showed significant gains in progression-free survival (the primary end point of the study) in all participants [22].

For patients presenting with EGFR mutations, TKIs are still the most effective combatant against NSCLC, and erlotinib has been among the most important developments in lung cancer treatment since the 1970s. Even as resistance to erlotinib and first-line TKIs arise, several new second-line agents are evolving to curb refractory disease.

Targeting VEGF & angiogenesis

Angiogenesis is the formation of new vasculature from existing blood vessels and is fundamental to physiological processes such as development, reproduction and wound healing [23]. In healthy patients, angiogenesis is limited to such discrete periods and a closely regulated balance exists between factors supporting and opposing the development of new vasculature [24]. However, angiogenesis is a hallmark of cancer and is essential for the growth and metastasis of tumors [7]. Circulating endothelial cells, along with cancerous cells, secrete proangiogenic factors – the most important of which is VEGF – to induce blood vessel formation [23]. Angiogenesis occurs by disruption of the endothelial cell basement membrane and extracellular matrix by proteolytic enzymes [25] and the release of membrane-sequestered angiogenic factors such as VEGF, basic FGF and TGF- β [26]. Within tumors, new blood vessels form from the existing vasculature, assisted by the circulating cells, such as bone marrow-derived endothelial progenitors, macrophages and fibroblasts [23]. Increased density of tumor vasculature has been demonstrated to correlate with advanced disease stage and poor prognosis in NSCLC, as has increased expression of proangiogenic factors [27].

The VEGF family of growth factors includes VEGF ligands that mediate angiogenesis and lymphangiogenesis through several receptors (VEGFRs) [28]. VEGFRs are present on the surfaces of tumors cells from a variety of cancers, including NSCLC [29]. A number of mAbs and VEGFR TKIs are both in use and under investigation for the treatment of lung cancer. VEGF (VEGF-A) is the most important proangiogenic factor, and bevacizumab (a mAb targeting it) was the first approved angiogenesis inhibitor [30]. Combined with conventional chemotherapy, bevacizumab displays anti-angiogenic properties and increases

overall survival first in colorectal [31] and then in NSCLC [32] patients. A recent Phase III study of cisplatin and gemcitabine with or without bevacizumab showed significantly improved progression-free survival upon administration of bevacizumab, and the drug is now arguably the most widely used targeted therapy in NSCLC treatment [33].

Sunitinib is a small-molecule TKI that targets a spectrum of membrane receptors, including VEGFR-1 and -2, as well as several others. A Phase II trial studying sunitinib activity in previously treated advanced NSCLC patients showed an 11.1% overall response rate [34]. Unfortunately, a number of studies investigating sunitinib in conjunction with other chemotherapeutics have demonstrated toxicity issues [35]. Current Phase III trials are investigating sunitinib alone and in conjunction with erlotinib in advanced NSCLC patients [302]. Like sunitinib, sorafenib is a TKI targeting a myriad of receptors including VEGFR-1 and -2. Despite successful Phase II trials, Phase III trials investigating sorafenib accompanied by cytotoxic chemotherapies have met limited success [35]. Other Phase III trials are ongoing [302]. Vandetanib (Zactima™, ZD6474 [AstraZeneca, Cheshire, UK]) inhibits the tyrosine kinase domain of VEGFR-2, has moderate anti-EGFR activity, and is in Phase III development for monotherapy and combination regimes treating NSCLC [36]. AMG 706 (motesanib [Amgen, Thousand Oaks, CA, USA]) is a potent kinase inhibitor of all known VEGFRs, PDGFR and Kit. It is undergoing Phase III evaluation for first-line treatment of advanced NSCLC in combination with paclitaxel and carboplatin [37]. AZD2171 (Recentin™, AstraZeneca) is an orally active TKI of all VEGFR subtypes. AZD2171 has completed Phase I evaluations in combination with gemcitabine and cisplatin as a first-line treatment for NSCLC and Phase II trials combined with pemetrexed for relapsed NSCLC. It is currently undergoing a Phase III randomized trial with carboplatin and paclitaxel to treat stage IIIB–IV NSCLC [38]. Axitinib (AG-013736) is a small-molecule TKI of VEGFR-1, -2 and -3, and demonstrates further activity against PDGFR-β and Kit. Axitinib has completed Phase II confirmation of activity in advanced NSCLC [39]. BIBF1120 is an angiogenesis-targeting TKI that inhibits VEGFRs, PDGFRs and FGFRs; it has shown low toxicity and considerable promise in Phase I/II trials and is now under Phase III investigation [40].

Targeting tumor vasculature with tumor-vascular-disrupting agents has been explored, but these agents, which induce acute collapse in vascular supply, have shown severe toxicities that have curtailed their use [41]. However, ASA404 (vadimezan) is a well-tolerated tumor-vascular-disrupting agent that was studied in Phase III trials in combination with carboplatin and paclitaxel, but the study was terminated after the primary end point of overall survival was not reached [303]. A Phase III trial studying ASA404 combined with docetaxel is ongoing [302].

Multi-targeted agents represent the new generation of targeted therapies. Given the established efficacy of erlotinib and bevacizumab, it is the natural next step to study their use in combination. A number of approaches to simultaneously inhibiting both EGFR and VEGF signaling are under scrutiny. A recent Phase I/II trial combining erlotinib and bevacizumab treating NSCLC supports their concurrent use [42]. Combined inhibition strategies appear to be well tolerated and show promise. However, the Phase III Bevacizumab plus Tarceva (erlotinib) (BeTa) trial did not reach its primary end point of longer overall survival for patients receiving both drugs (9.3 vs 9.2 months for erlotinib plus placebo; hazard ratio [HR]: 0.97; 95% CI: 0.8–1.18; $p = 0.75$). The BeTa trial did show that combination therapy resulted in doubled progression-free survival time (3.4 vs 1.7 months for erlotinib plus placebo; HR: 0.62; 95% CI: 0.52–0.75; $p < 0.0001$) [43]. It is possible that the heterogeneous nature of NSCLC limits the ability to detect benefits from inhibition of a specific target in an unselected patient population [42].

Targeting IGF-1R

The IGF pathway, implicated in the regulation of fetal development, tissue growth and metabolism, is comprised of two main active receptors (IGF receptor [IGF-1R] and insulin receptor [IR]) and three main ligands (IGF-1, IGF-2 and insulin) [44]. The receptors exist either as homodimers or as hybrid heterodimers of IGF-1R and IR, and are activated upon ligand binding [45]. In the presence of oncogenic signals, IGF-1R promotes tumor survival and growth. IGF-1R is a receptor tyrosine kinase comprised of two extra cellular subunits bonded to two single-pass membrane-spanning subunits, where cytoplasmic tyrosine kinase activity takes place [45]. Activation of the receptor results in phosphorylation of insulin receptor substrate proteins (IRS-1–4), which in turn initiate further signaling cascades. IRS-1 has known associations with proliferation, and IRS-2 with metastatic behavior [46]. IRS-1 also activates PI3K, which results in activation of Akt. In turn, Akt activation leads to both inhibition of pro-apoptotic factors and to increased expression of anti-apoptotic proteins such as Bcl-2 [47]. Because insulin is a ligand for IGF-1R, an anticipated (and noted) toxicity for IGF-1R inhibitors is hyperglycemia [48]. IGF-1 is a polypeptide hormone structurally similar to human pro-insulin, and is involved in somatic growth [49]. IGF-2 is present in the circulation at two-to-three-times higher levels than IGF-1, and is the predominant IGF in adults [50]. IGF-1R has a 15-20-fold higher affinity for IGF-1 than IGF-2, but IGF-2 possesses greater binding potential across different receptors and therefore has a broader range of biologic functions. Elevated IGF levels are found in the blood of lung cancer patients, in addition to elevated levels in tumor (versus normal lung) tissue [51]. Increased IGF-1 plasma levels have been associated with increased risk of lung cancer [52]; therefore, inhibition of the IGF pathway is a new target for both stand-alone therapies as well as another target for existing therapeutic regimens. In addition, the IGF and EGF pathways interact on multiple levels and evidence suggests that IGF-1R mediates resistance to anti-EGFR therapies by keeping the PI3K–Akt pathway activated [53]. Although IGF-1R expression does not appear to be associated with gefitinib resistance, high coexpression of both IGF-1R and EGFR seems to be a significant prognostic indicator of worse disease-free survival [54,55].

There are two main groups of IGF-1R inhibitors: small-molecule TKIs and mAbs directed against the extracellular domain [45]. The design of IGF-1R TKIs is complicated by 85% homology between the receptors kinase domain and that of the IR [56]. However, there are a handful of TKIs that exploit subtle differences between the two receptors and these are in pre clinical and early clinical trials to study their efficacy in combating advanced solid tumors [57].

Figitumumab (CP-751871) is a fully human IgG2 mAb directed against IGF-1R, which is currently under investigation. It selectively binds IGF-1R and prevents other ligands from binding to the receptor [58]. Of particular interest is that figitumumab appears to have a high degree of efficacy in patients with squamous cell histology, and it is the first drug reporting clinical success in non-adenocarcinoma NSCLC [59]. A recent Phase III trial was commenced studying the simultaneous inhibition of EGFR and IGF-1R signaling, using both erlotinib and figitumumab in non-adenocarcinoma NSCLCs, but was discontinued in March 2010 after an independent Data Safety Monitoring Committee concluded that the study was unlikely to demonstrate a statistically significant improvement in the primary end point of overall survival compared with erlotinib alone [304]. Another Phase III study evaluating figitumumab in conjunction with carboplatin and paclitaxel was also halted in December 2009 after some patients receiving all three drugs experienced severe adverse events, including death [305].

A number of other anti-IGF-1R mAbs are in early clinical development as first- and second-line treatments for advanced NSCLC, including R1507, MK-0646, AMG-479 and cixutumumab. Some of the studies are also enrolling exclusively squamous NSCLC patients in order to elucidate whether the drugs act specifically on that histology [302].

Targeting the PI3K–Akt pathway

The PI3Ks are a family of enzymes activated by receptor tyrosine kinase signaling, and the PI3K–Akt signaling pathway is crucial for the growth and survival of a number of cancers. The serine/threonine kinase Akt, one of the most important downstream targets of PI3K, can be activated via tyrosine kinase receptors and G-protein-coupled receptors [60]. Akt transmits oncogenic signals and mediates cellular responses ranging from cell growth, transformation, differentiation and motility to survival [61]. The PI3K–Akt pathway is implicated in the regulation of a number of cellular pathways, including the phosphorylation-dependent inhibition of pro-apoptotic proteins such as Bad, Bix and Bid, and the activation of anti-apoptotic proteins such as Bcl-xL [62]. PI3K inhibitors (e.g., LY294002) increase sensitivity to apoptosis-promoting therapeutics in NSCLC cell lines, and prevent growth and colony formation *in vitro* [63]. The PI3K pathway inhibitor, GDC-0941 (Genentech, San Francisco, CA, USA), is in Phase Ib trials for combined therapy with carboplatin and paclitaxel with and without bevacizumab. Downstream, the PI3K–Akt pathway is mediated by the serine/threonine kinase mTOR, which regulates basic cellular functions such as cellular growth and proliferation. Rapamycin (sirolimus; Rapamune® [Wyeth, Madison, NJ, USA]) is an mTOR inhibitor that has completed Phase II studies [64]. The related mTOR inhibitor everolimus (RAD001; Zortress® [Novartis, Basel, Switzerland]) has completed Phase I and II studies for use as both a monotherapy and with combination regimes [302]. The only available data addressing the efficacy of PI3K pathway inhibitors for the treatment of NSCLC come from a completed Phase II trial of everolimus as a monotherapy for heavily pretreated advanced-stage NSCLC patients. This study indicates that the drug is well tolerated and shows moderate clinical activity [65]. Meanwhile, several studies have demonstrated that downregulation of PI3K is necessary for effective therapies [66,67].

Targeting the TRAIL apoptotic pathway

TNF-related apoptosis-inducing ligand (TRAIL) receptor-targeting agents depend on their ability to induce apoptosis in tumor cells [68]. TRAIL activates apoptosis by binding to certain transmembrane receptors, resulting in the trimerization of these receptors and formation of a death-inducing signaling complex (DISC) activated by cleaving procaspase-8 from Fas-associated death domain (FADD). Apoptosis can be suppressed by the recruitment of the cellular FLICE-inhibitory protein to DISC, thereby preventing caspase-8 binding and activation [69]. Although the extrinsic apoptotic pathway initiated by caspase-8 activation is sufficient for irreversible apoptosis activation via caspase-3 activation, in NSCLC cells, cross-activation of the intrinsic route via caspase-8-dependent Bid cleavage becomes necessary [70]. Cleaved Bid, or truncated (t)Bid, interacts with Bcl-2 family proteins to activate the apoptosome. In addition to the primary DISC, a secondary signaling complex can also form, which activates several cell proliferation-promoting kinase pathways, including activation of NF-κB [71]. Since the discovery of the TRAIL apoptotic pathway, several agents targeting one or both of the functional TRAIL receptors (TRAIL-R1 and TRAIL-R2) have been developed. These agents include both recombinant human TRAIL (rhTRAIL)-derived selective mutant forms designed to preferentially bind one of the two receptors [72–75] as well as agonistic mAbs targeted against one receptor or the other [76–79].

The p53 pathway, which is important in mediating chemotherapy- and radiotherapy-induced cell death, is inactivated in more than 50% of NSCLCs [80]. Moreover, many NSCLC cells subjected to chemotherapy possess a defect in DNA damage-dependent caspase-9 activation, which could result in chemotherapy resistance [81]. Because TRAIL initiates apoptosis via caspase-8, it is less dependent on wild-type p53 or caspase-9 mechanisms. Interestingly, IHC studies demonstrate that TRAIL-R1, TRAIL-R2 and TRAIL are expressed in 99, 82 and 91% of NSCLCs, respectively [82]. Preclinical *in vitro* and *in vivo* models of TRAIL-targeting agents have demonstrated that NSCLC cell lines, and xenograft and orthotopic mice are extremely vulnerable to apoptosis by this route [79,83].

Studies indicate that approximately half of NSCLC cell lines possess intrinsic resistance to apoptosis via TRAIL receptor targeting. Several mechanisms of TRAIL resistance have been observed, including inhibition and defects at the receptor and DISC level to suppress downstream signaling in the pathway [84]. The frequency of intrinsically TRAIL-resistant tumors necessitates strategies for sensitizing tumors to TRAIL-induced apoptosis. Combining TRAIL receptor-targeting therapies with standard chemotherapy and radiotherapy regimens has been found to greatly increase anti-tumor activity in NSCLC models. Bortezomib (PS-341; Velcade®, Millennium Pharmaceuticals, MA, USA) is a proteasome inhibitor approved as a therapy for multiple myeloma patients and has been extensively studied in NSCLC. Bortezomib binds reversibly to the 26S proteasome and thereby inhibits the degradation of several proteins involved in cell cycle regulation and apoptosis. Combining bortezomib with TRAIL-targeting agents is driven by the rationale that the former can inhibit NF-κB signaling, a key mechanism that can cause TRAIL resistance [85]. Also of interest is combining EGFR inhibitors with TRAIL receptor targeting, since activation of downstream Akt pathways is correlated with TRAIL resistance in NSCLC. Combining EGFR inhibitor PD153035 with rhTRAIL appears to sensitize A549 cells to apoptosis [86].

At present, the only clinical trial with rhTRAIL (AMG 951, Amgen) in NSCLC is studying it in combination with paclitaxel and carboplatin, with and without bevacizumab. So far, the Phase IB study showed no dose-limiting toxicities and an overall response rate of 56%, and a randomized Phase II trial in previously untreated patients with advanced NSCLC is underway [87]. Mapatumumab is a fully humanized anti-TRAIL-R1 mAb. It has been studied as a single agent in Phase II trials with heavily pretreated advanced NSCLC patients and appears to be well tolerated with only mild adverse events [77]. However, a recently completed Phase II trial of mapatumumab combined with paclitaxel and carboplatin as first-line treatment in advanced NSCLC failed to produce positive outcomes [88]. This trial was based in an unselected population of patients, so perhaps future studies targeting a more restricted population may prove more fruitful. There are also several studies with agonistic TRAIL-R2 antibodies as both mono and combination therapies. AMG 655 (Amgen) is an anti-TRAIL-R2 antibody used as a single agent in a Phase I study that showed a NSCLC patient with a 46% reduction in tumor volume [89]. AMG 655 is currently being combined with paclitaxel and carboplatin as a first-line treatment in a Phase Ib/II trial studying NSCLC [90].

TNF-related apoptosis-inducing ligand receptor targeting provides an interesting and rational approach for treating NSCLC. Although half of NSCLC cell culture models demonstrate resistance to TRAIL-induced apoptosis, combining treatment with new and existing therapeutic regimens can greatly enhance anti-tumor activity. Also of interest is studying whether TRAIL has an apoptosis-inducing effect on cancer stem cells.

Targeting heat-shock proteins

Heat-shock proteins (Hsps) are so named because they are often produced in times of cellular stress, in response to temperature or anaerobic shock. However, they are also expressed under normal conditions and act as cellular chaperones by traveling alongside other proteins and assisting in their proper folding and stabilization, as well as helping to sequester damaged proteins targeted for degradation [5]. However, acting as chaperones, Hsps also aid the carcinogenic activities of oncogenic proteins. Thus, inhibition of Hsp activity interferes with oncoprotein activity and can disrupt the carcinogenesis cascade [91].

Hsp90 is a ubiquitously expressed protein necessary for a plethora of essential cellular functions, including signaling, proliferation and survival [92]. It is also responsible for the conformational maturity of a number of enzymes implicated in the various carcinogenesis pathways leading to the six hallmarks of cancer [7], for example, the Src family kinases, receptor tyrosine kinases such as EGFR, mutant p53, steroid hormone receptors and MET, among others [93]. Geldanamycin compounds are long-studied Hsp90 inhibitors. Although the extremely low water solubility of geldanamycins has hindered their development as effective therapeutics, their 17-amino derivatives, particularly 17-AAG, have shown significant therapeutic promise [94]. They have reduced hepatotoxicity (another shortcoming of geldanamycin), but their poor solubility has also proven a pharmaceutical hurdle [5,95]. SNX-5422 is an Hsp90 inhibitor with a similar pharmacological profile to 17-AAG and is in Phase I trials for solid tumors and other cancers [96]. Another Hsp90 inhibitor, IPI-504 (Infinity Pharmaceuticals, MA, USA), is about to be tested in a new Phase II clinical trial for NSCLC patients exhibiting an ALK mutation (ALK mutations are discussed later in this article), and STA-9090 is in Phase II trials for late-stage NSCLC [302].

Heat-shock protein 70 protects cells from environmental stress damage by preventing misfolding, aggregation and denaturation of cytoplasmic proteins. It targets cells for cytolytic degradation and it inhibits apoptotic pathways [91]. Membrane-bound Hsp70 binds CD94 receptors on the surface of natural killer cells, causing them to secrete granzyme B, inducing apoptosis [97]. This event can be further exploited for therapeutic benefit.

Heat-shock protein 90 appears to interact with hypoxia-inducible factor-1 (HIF-1 α) protein. The HIF family of transcription factors consist of a constitutively expressed β -subunit whose mRNA and protein levels are not regulated by oxygen levels [98] and three α -subunits: HIF-1 α , HIF-2 α and HIF-3 α (all of which are closely regulated by oxygen levels within the cell). HIF-1 α is ubiquitously expressed in human tissue and is believed to be the primary factor governing general response to hypoxia [99]. Under normal oxygen conditions, HIF-1 α is polyubiquitinated and proteosomally degraded, but under hypoxic conditions the protein heterodimerizes with its β -subunit and initiates transcription of target genes [98]. HIF-1 α has a significant role in mediating blood vessel maturity [100] and its overexpression is observed in a number of solid tumors, including breast, prostate, brain, lung, and head and neck cancers [101]. HIF-1 α activation can be induced by various cytokines and growth factors, as well as by loss of tumor suppressor gene or gain of oncogene expression. Tumors treated with anti-VEGF therapy often develop resistance by selection of hypoxia-resistant cells, suggesting that targeting HIF-1 α may aid the targeting of resistant cells. In addition, HIF-1 activity is the dominant tumor response to irradiation and causes radioprotection of tumor vasculature [31]. Radiation-induced HIF-1 α protein expression occurs through two distinct pathways: activation of PI3K/Akt/mTOR [32] and augmentation of the Hsp90–HIF-1 α interaction. The latter interaction was only enhanced in radioresistant cells [44]. Blocking HIF-1 enhances the destruction of vasculature and significantly enhances radiosensitivity [31]. Inhibition of Hsp90 using 17-AAG suppressed HIF-1 α expression and

decreased the survival and angiogenesis of radioresistant lung cancer cells *in vitro* [45]. Thus, the rationale for targeting Hsp90 also includes combining therapeutics with radiation therapy.

Targeting histone deacetylases

Gene expression via epigenetic modification is a key regulatory process in the cell [102]. This regulation occurs in the context of DNA being packaged in the cell so that it is coiled around a core comprised of eight histone proteins, the N-terminals of which extend out through the DNA strand [103]. Cell development and regulation rely heavily on histone modification, and it is implicated in the pathobiology of cancer as well as other diseases [104]. Modification occurs post-translationally by acetylation, methylation and phosphorylation of the N-terminal tails, thereby structurally rearranging the spatial relation of the tails in relation to the DNA strand and giving transcription factors access to gene promoter regions [105,106]. Conversely, deacetylation, demethylation and dephosphorylation of the histone tails effectively decrease access to these promoter regions [106]. Histone acetylation, the best understood of the aforementioned three post-translational modifications, is mediated by histone acetyltransferases [107], and the acetyl groups are removed by histone deacetylases (HDACs) [108].

Histone deacetylase inhibitors cause cell cycle arrest and apoptosis by blocking the deacetylation function of HDAC, thereby upregulating the intrinsic apoptotic pathway [109,110]. Moreover, hyperacetylation of histone tails stabilizes the p53 protein and promotes both cell cycle arrest as well as the expression of proapoptotic factors [111]. HDACs are involved with hypoxia-induced angiogenesis [112] and indirectly regulate HIF-1 α activity under hypoxic conditions [113].

Although HDAC inhibitors are relatively non-toxic to normal tissue, but they not only exhibit selective cytotoxicity against cancer cells, they also enhance the cytotoxic effects of radiation and synergistically promote the effects of chemotherapy. CI-994 is an orally available inhibitor of histone deacetylation and cellular proliferation at G1–S phase transition, and has demonstrated anti-tumor activity both *in vitro* and *in vivo* in randomized Phase II trials [302]. CI-994 has also been evaluated with and without gemcitabine in a randomized Phase III trial in lung cancer patients, with results still pending [302]. Vorinostat (suberoylanilide hydroxamic acid), an inhibitor of enzymatic activity of HDAC1, HDAC2, HDAC3 (class I) and HDAC6 (class II), was used in a Phase III clinical trial that was terminated when the primary end point of improved overall survival was not reached. However, a Phase I/II trial of vorinostat in combination with erlotinib for patients showing disease progression upon administration of erlotinib alone is ongoing, and a new trial is recruiting patients without prior radiotherapy or chemotherapy to validate the molecular targets of vorinostat in resectable stage I–III NSCLCs [302].

Panobinostat (LBH589, Novartis) sensitizes human NSCLC cell lines (H23 and H460) to radiation-induced DNA double-strand breaks and, in combination with radiotherapy, demonstrated tumor growth delay in human lung cancer xenografts in nude mice [114]. EGFR mutation status is possibly predictive of outcome with panobinostat and even other HDAC inhibitors [115]. Panobinostat is currently in Phase I clinical trials to determine its pharmacokinetics and safety in advanced solid tumors [302]. Preliminary clinical and preclinical studies indicate that HDAC inhibitors are more effective combined with chemotherapeutics than as single agents [110].

Targeting stem cell pathways

Increasing evidence suggests that abnormal activation of tightly controlled developmental pathways can lead to cancer neoplasia. In particular, the Hedgehog (Hh) and Wntless (Wnt) pathways have been shown to play a role in both lung organogenesis as well as in carcinogenesis [116]. A hurdle in the design of current treatments of lung cancer is the intrinsic resistance of the 'stem-like' progenitor cells following therapy, resulting in disease recurrence and poor patient prognosis. The theory of 'cancer stem cells' (CSCs) has been gaining traction as the number of cancers, including NSCLC, showing attributes of a 'stem cell compartment' increases [117-119]. By identifying and exploiting the discrete therapeutic vulnerabilities of these CSC populations, better survival rates for patients may be achieved.

The Wnt signaling pathway was so named after the gene responsible for phenotypic winglessness in drosophila. Activation of the signal transduction pathway is caused by secreted Wnt ligands that trigger changes in gene expression as well as in cell behavior, adhesion and polarity. Although Wnt signaling has been described in at least three pathways [120], the most fully characterized Wnt pathway is that of the canonical signaling cascade: Wnt ligands bind to two families of cell-surface receptors, the frizzled (Fz) receptors and the low-density lipoprotein receptor-related proteins, and activate target genes through stabilization of nuclear β -catenin [121]. Wnt appears to be necessary for stem cell maintenance and is activated upstream of β -catenin in NSCLC. Repression of Wnt pathway antagonists such as Wnt inhibitory factor (WIF-1) and abnormal overexpression of effectors such as dishevelled (DVL) are essential to the development of lung cancer. Both the fetal lung and NSCLC highly express Wnt-2, and NSCLC cell lines – as well as primary lung cancers – show overexpression of Wnt-1 [122]. More specifically, Wnt-1 signaling appears to inhibit apoptosis by activating β -catenin/T-cell factor (TCF)-mediated transcription [123]. Blockading critical components of the Wnt pathway is a potential strategy for inhibiting stem-like pathways to carcinogenesis. Using a siRNA to block Wnt signaling reduced tumor growth and induced apoptosis *in vivo*, as did using a specific mAb against Wnt-1 to the same end [122]. The DVL proteins are downstream effectors of Wnt signaling. DVL-3, which is overexpressed in 75% of NSCLCs, plays a significant role in both Wnt signaling and subsequent tumor cell proliferation in lung cancers. Growth inhibition was observed in the presence of siRNA treatment against DVL [124]. WIF-1 has been shown to inhibit growth in NSCLC cell lines both *in vivo* and *in vitro* [125], and silencing of WIF-1 via promoter hypermethylation is known to anomalously activate Wnt signaling in lung cancer [126]. A number of small-molecule inhibitors of the Wnt pathway, namely of β -catenin and TCF, are under investigation in preclinical studies [127,201-203]. ICG-001 is a small molecule that downregulates β -catenin and TCF and induces apoptosis in colon cancer, both *in vitro* and *in vivo* [128]. However, specifically blocking the β -catenin/TCF interaction has proven difficult because β -catenin interacts with other binding partners such as adenomatous polyposis coli, axin and E-cadherin along the same interface as its interaction with TCF [129].

Also indispensable for normal mammalian development is the Hh pathway. Activation of the signaling cascade occurs through binding of the Sonic hedgehog (Shh), Indian hedgehog (Ihh), and Desert hedgehog (Dhh) morphogens to their receptor Patched, thereby inhibiting the repression of Smoothened and regulating the downstream transcription factors (Gli-1, Gli-2 and Gli-3). Hh-mediated epithelial–mesenchymal tissue interactions direct the branching of pulmonary buds during murine fetal development. Shh expression peaks during embryogenesis, but decreases before birth, remaining detectable throughout the postnatal epithelium [130]. Shh-null mice exhibit lung buds without airway branching, with obviously dire consequences for viability, and transgenic over-expression of Shh leads to absence of

alveoli and hyperproliferation of epithelial and mesenchymal pulmonary cells [131]. Small-cell lung cancer cell lines, which maintain components of the Hh pathway, are inhibited by cyclopamine (a steroidal alkaloid inhibitor of Smoothened, and therefore Hh), but the results were not observed in NSCLC [132,133]. GDC-0449 (Genentech) is a small-molecule inhibitor of the Hh pathway currently in clinical trials, but not in lung cancer as of yet [134,302]. After successfully completing a Phase I study in basal cell carcinoma, GDC-0449 was determined to be safe for human use and showed modest activity in the patient cohort [134]. Now a number of Phase II trials are investigating the drug's efficacy in treating refractory basal cell carcinoma, advanced-stage colorectal cancer and ovarian cancer [302]. Robust identification of putative CSCs in lung cancer will give rise to novel signal transduction pathway targets in combating lung cancer.

EML4-ALK

In 2007, a fusion of the anaplastic lymphoma kinase (ALK) with the echinoderm microtubule-associated protein-like 4 (EML4) was described in Japanese NSCLC patients [135]. Studies, mostly involving East Asian patients, report that 3–13% of lung tumors harbor EML4–ALK fusions [135,136] – a statistic that can be extrapolated to 5% of all NSCLC cases (or 70,000 patients per year, worldwide). The fusion protein occurs most frequently in a unique subgroup of patients similar to those likely to harbor EGFR mutations, such as female gender, Asian ethnicity and never- or former light-smoker status [136]. However, with few exceptions, EML4–ALK and EGFR mutations appear to be mutually exclusive [137]. In particular, EML4–ALK translocations appear to occur in younger patients with more advanced disease than in those with EGFR mutations [137]. Because the ALK tyrosine kinase activity is necessary for oncogenicity, ALK TKIs are under evaluation in preclinical *in vitro* and *in vivo* models. These preliminary studies show that ALK inhibitors lead to apoptosis and tumor shrinkage; this is also confirmed by clinical studies. A Phase I trial of PF-02341066 (crizotinib, initially designed as an inhibitor of MET) showed a 53% response rate and 79% disease control rate (partial response and stable disease) in EML4–ALK NSCLC patients [138]. These overwhelmingly positive results led to a randomized Phase III trial of crizotinib combined with standard pemetrexed or docetaxel chemotherapy in second-line EML4–ALK NSCLC as well as a Phase II trial of crizotinib as a single agent in EML4–ALK NSCLC patients not otherwise eligible for the Phase III trial [302]. This latter Phase II trial demonstrated striking activity of crizotinib in ALK-positive patients: the overall response rate was 57%, and although median progression-free survival has not been reached, the 6-month progression-free survival rate was 72% [139]. EML4–ALK NSCLC represents a unique subset of lung cancer patients for whom a specialized subset of therapies may prove effective. It is of particular note that in the 3 years since the translocation's discovery, therapies uniquely directed for these patients are already meeting with dramatic success in clinical trials.

Expert commentary

At 15%, the dismal 5-year survival rate of lung cancer patients has changed little since the 1970s. Standard chemotherapeutic regimens have reached a plateau with regard to improving disease progression and overall survival. Elucidation of the complex mechanisms underlying respiratory carcinogenesis offers a plethora of attractive targets for specialized therapeutic regimes. New small-molecule inhibitors and effectors are being designed to target a number of important signal transduction pathways implicated in lung cancer (Figure 1 & Table 1).

The efficacy of different targeted therapies varies among lung cancer histologies, but our scant knowledge regarding the molecular basis of the histologies prevents us from

effectively stratifying patients based upon the likelihood of them benefiting from a certain therapy. In recent years, especially with enhanced feasibility of genome-wide studies, prognostic gene signatures are cropping up, characterizing patients on the basis of disease risk, histology and other markers. EGFR mutation status and EML4–ALK4 trans-location status are just early examples of genetic profiles known to affect outcomes and response to specific therapies. As we better understand the molecular profiles of patients, targeted therapies are becoming increasingly streamlined towards use in patient cohorts most likely to benefit from them. The stunning success of drugs targeting EML4–ALK and, especially, EGFR mutations, highlights the imperative to implement universal screening processes to identify these mutations and maximize the number of patients who are able to benefit from these targeted therapies.

Furthermore, with each generation of targeted agents, a new generation of drug resistance mechanisms arises. It is becoming increasingly apparent that targeting a single pathway is an ineffective remedy to rapidly evolving lung cancers. Instead, focus is shifting to the development of combination therapies, such as erlotinib and bevacizumab combined as a first-line treatment, and agents that work synergistically with existing regimens (for example targeting Hsp90 in conjunction with radiation treatment) (Table 1). As is, the signaling transduction pathways discussed here possess significant overlap, for example, the PI3K–Akt pathway is directly implicated in IGF, TRAIL and HIF signaling, among others, and there is a great deal of crosstalk (Figure 1). Therefore, combining regimens of targeted agents is of paramount importance.

Five-year view

In breast cancer, Genomic Health Inc. (CA, USA) has a paraffin-based quantitative PCR assay, OncotypeDx[®], which assigns breast cancer patients a risk score based upon which the course of adjuvant treatment can be decided [140]. Similar technologies may stratify early-stage lung adenocarcinoma patients based upon recurrence risk and survival prognosis, and a number of groups have already proposed prognostic gene markers for such an assay [141–143]. Currently, the standard of care for stage I adenocarcinoma patients is surgical resection without adjuvant chemo therapy [301], but approved prognostic assays can be used to recruit early-stage patients into clinical trials for better targeted chemotherapies. As new gene signatures are discovered, late-stage patients can be grouped based on tumor histology into drug trials for their more specific phenotypes.

Furthermore, even though we already understand that factors such as EGFR expression and mutation status correlate with prognosis and drug response, the technologies used to identify these factors (IHC and sequence analysis) are either too crude (IHC) or time-consuming/expensive (sequence analysis) for use in identifying the course of first-line treatment. Over the next few years, as costs for sequence and mutational analysis fall, screening for EGFR (and other genes of interest) will become routine. Kim *et al.* describe a cost-effective denaturing high-performance liquid chromatography plate for high-throughput adenomatous polyposis coli mutation screening in colon cancer biopsies [144], and such a technology could easily become applicable in the treatment of lung cancer.

Lastly, the heterogeneous nature of lung cancers makes it difficult for us to interpret data on highly specialized therapies used in very diverse patient populations. As we become better able to stratify patients based on a refined understanding of their histology, we can revisit previously discarded targeted therapies to see if their efficacy is applicable to a more constrained population. The next few years will herald the advent of personalized medicine as treatments become more uniquely tailored to each lung cancer patient's individual biology.

Key issues

- Prolonged exposure to single-agent targeted therapies results in drug resistance.
- There is a great deal of crosstalk among various signaling pathways, making it necessary to target multiple pathways at a time.
- The heterogeneity of lung cancers renders difficult our understanding of how a specialized agent acts in a varied patient population. We need better methods of identifying and stratifying lung cancer histology to study the benefits of targeted agents on controlled populations.
- Cancer therapeutics is rapidly progressing towards personalized medicine and patients' treatment regimens are becoming increasingly based upon their own unique biology.

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References

Papers of special note have been highlighted as:

- of interest
 - of considerable interest
- 1•. American Cancer Society. Cancer Facts & Figures 2009. American Cancer Society; Atlanta, GA, USA: 2009. Comprehensive summary of the current status of various cancer types, updated annually by the American Cancer Society
 2. National Cancer Institute. SEER Cancer Statistics Review 1975–2004. National Cancer Institute; Bethesda, MD, USA: 2008. Non-small cell lung and bronchus cancer (invasive) survival rates, by race, sex, diagnosis year, state and age.
 3. Greenlee R, Murray T, Bloden S, Wingo PA. Cancer statistics. CA Cancer J. Clin 2000;50:7–33. [PubMed: 10735013]
 4. Bunn PJ, Kelly K. New combinations in the treatment of lung cancer: a time for optimism. Chest 2000;117:138–143.
 5. Zhang Q, Feng W, Zhou H, Yan B. Advances in preclinical small molecules for the treatment of NSCLC. Expert Opin. Ther. Patents 2009;19:741–751.
 6. Carpenter G, Cohen S. Epidermal growth factor. J. Biol. Chem 1990;265:7709–7712. [PubMed: 2186024]
 7. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57–70. [PubMed: 10647931]
 8. Ciardiello F, Tortora G. A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. Clin. Cancer Res 2001;7:2958–2970. [PubMed: 11595683]
 - 9•. Gazdar A. Personalized medicine and inhibition of *EGFR* signaling in lung cancer. N. Engl. J. Med 2009;361:1018–1020. Summarizes the current status of targeted inhibition of the EGF receptor in the treatment of non-small-cell lung cancer. [PubMed: 19692681]
 10. Azim HA Jr, Ganti AK. Targeted therapy in advanced non-small cell lung cancer (NSCLC): where do we stand? Cancer Treat. Rev 2006;32:630–636. [PubMed: 17034953]

11. Sequist L. Second-generation epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer. *Oncologist* 2007;12:325–330. [PubMed: 17405897]
12. Taron M, Ichinose Y, Rossell R, et al. Activating mutations in the tyrosine kinase domain of the epidermal growth factor receptor are associated with improved survival in gefitinib-treated chemorefractory lung adenocarcinomas. *Clin. Cancer Res* 2005;11:5878–5885. [PubMed: 16115929]
13. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med* 2005;350:2129–2139. [PubMed: 15118073]
14. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N. Engl. J. Med* 2010;362:2380–2388. [PubMed: 20573926]
15. Han S, Kim TY, Hwang PG, et al. Predictive and prognostic impact of epidermal growth factor receptor mutation in non-small-cell lung cancer patients treated with gefitinib. *J. Clin. Oncol* 2005;23:5900–5909. [PubMed: 16043828]
16. Engelman J, Jänne P. Mechanisms of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer. *Clin. Cancer Res* 2008;14:2895–2899. [PubMed: 18483355]
17. Cappuzzo F, Marchetti A, Skokan M, et al. Increased *MET* gene copy number negatively affects survival in surgically resected non-small-cell lung cancer patients. *J. Clin. Oncol* 2009;27:1667–1674. [PubMed: 19255323]
18. Schiller JH, Akerley W, Brugger W, et al. Results from ARQ 197–209: a global randomized placebo-controlled Phase II clinical trial of erlotinib plus ARQ 197 versus erlotinib plus placebo in previously treated EGFR inhibitor-naïve patients with locally advanced or metastatic non-small-cell lung cancer (NSCLC). *J. Clin. Oncol* 2010;28(18 Suppl.) abstract LBA 7502.
19. Sequist LV, Besse B, Lynch TJ, et al. Neratinib, an irreversible Pan-ErbB receptor tyrosine kinase inhibitor: results of a Phase II trial in patients with advanced non-small-cell lung cancer. *J. Clin. Oncol* 2010;28:3076–3083. [PubMed: 20479403]
20. Riely G. Second-generation epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer. *J. Thorac. Oncol* 2008;3:146–149.
21. Takezawa K, Okamoto I, Tanizaki J, et al. Enhanced anticancer effect of the combination of BIBW2992 and thymidylate synthase-targeted agents in non-small cell lung cancer with the T790M mutation of epidermal growth factor receptor. *Mol. Cancer Ther* 2010;9:1647–1656. [PubMed: 20530710]
22. Boyer MJ, Blackhall FH, Park K, et al. Efficacy and safety of PF299804 versus erlotinib (E): a global, randomized Phase II trial in patients (pts) with advanced non-small cell lung cancer (NSCLC) after failure of chemotherapy (CT). *J. Clin. Oncol* 2010;28(18 Suppl.) abstract LBA 7523.
- 23••. Folkman J. Angiogenesis: an organizing principle for drug discovery? *Nat. Rev. Drug Discov* 2007;6:273–286. This excellent article by the pioneer in the field of angiogenesis gives a comprehensive review of the important roles of angiogenesis in tumorigenesis and as a therapeutic target. [PubMed: 17396134]
24. Jain R. Normalization of tumor vasculature: an emerging concept in angiogenic therapy. *Science* 2005;307:58–62. [PubMed: 15637262]
25. Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. *Nat. Rev. Cancer* 2003;3:401–410. [PubMed: 12778130]
26. Kalluri R. Basement membranes: structure, assembly and role in tumour angiogenesis. *Nat. Rev. Cancer* 2003;3:422–433. [PubMed: 12778132]
27. Fontanini G, Lucchi M, Vignati S, et al. Angiogenesis as a prognostic indicator of survival in non-small cell lung carcinoma: a prospective study. *J. Natl Cancer Inst* 1997;89:881–886. [PubMed: 9196255]
28. Hicklin D, Ellis LM. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. *J. Clin. Oncol* 2005;23:1011–1027. [PubMed: 15585754]
29. Fong G, Rossant J, Gertsenstein M, Bretiman ML. Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. *Nature* 1995;376:66–70. [PubMed: 7596436]

30. Ferrara N, Hillan KJ, Gerber HP, Novotny W. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. *Nat. Rev. Drug Discov* 2004;3:391–400. [PubMed: 15136787]
31. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N. Engl. J. Med* 2004;350:2335–2342. [PubMed: 15175435]
32. Sandler A, Gray R, Perry MC, et al. Paclitaxel–carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N. Engl. J. Med* 2006;355:2542–2540. [PubMed: 17167137]
33. Reck M, von Pawel J, Zatloukal P, et al. Phase III trial of cisplatin plus gemcitabine with either placebo or bevacizumab as first-line therapy for nonsquamous non-small-cell lung cancer: AVAIL. *J. Clin. Oncol* 2009;27:1227–1234. [PubMed: 19188680]
34. Socinski MA, Novello S, Brahmer JR, et al. Multicenter, Phase II trial of sunitinib in previously treated, advanced non-small-cell lung cancer. *J. Clin. Oncol* 2008;26:650–656. [PubMed: 18235126]
35. Pal SK, Figlin RA, Reckamp K. Targeted therapies for non-small cell lung cancer: an evolving landscape. *Mol. Cancer Ther* 2010;9:1931–1944. [PubMed: 20571071]
36. Amir E, Hughes S, Blackhall F, et al. Targeting blood vessels for the treatment of non-small cell lung cancer. *Curr. Cancer Drug Targets* 2008;8:392–403. [PubMed: 18690845]
37. Blumenschein G Jr, Reckamp K, Stephenson GJ, et al. Phase 1b study of motesanib, an oral angiogenesis inhibitor, in combination with carboplatin/paclitaxel and/or panitumumab for the treatment of advanced non-small cell lung cancer. *Clin. Cancer Res* 2010;16:279–290. [PubMed: 20028752]
38. Goss G, Arnold A, Shepherd FA, et al. Randomized, double-blind trial of carboplatin and paclitaxel with either daily oral cediranib or placebo in advanced non-small-cell lung cancer: NCIC clinical trials group BR24 study. *J. Clin. Oncol* 2010;28:49–55. [PubMed: 19917841]
39. Schiller J, Larson T, Ou SI, et al. Efficacy and safety of axitinib (AG-013736; AG) in patients with advanced non-small cell lung cancer (NSCLC): a Phase II trial. *J. Clin. Oncol* 2007;25(18 Suppl.) abstract 7507.
40. Reck M. BIBF 1120 for the treatment of non-small cell lung cancer. *Expert Opin. Investig. Drugs* 2010;19:789–794.
41. Head M, Jameson MB. The development of tumor vascular-disrupting agent ASA 404 (vadimezan, DMXAA): current status and future opportunities. *Expert Opin. Investig. Drugs* 2010;19:295–304.
42. Pennell N, Lynch TJ Jr. Combined inhibition of the VEGFR and EGFR signaling pathways in the treatment of NSCLC. *Oncologist* 2009;14:399–411. [PubMed: 19357226]
43. Hainsworth J, Herbst R. A Phase III, multicenter, placebo-controlled, double-blind, randomized clinical trial to evaluate the efficacy of bevacizumab (Avastin) in combination with erlotinib (Tarceva) compared with erlotinib alone for treatment of advanced non-small cell lung cancer after failure of standard first-line chemotherapy. *J. Thorac. Oncol* 2008;3:S302.
44. Clemmons D. Modifying IGF-1 activity: an approach to treat endocrine disorders, atherosclerosis, and cancer. *Nat. Rev. Drug Discov* 2007;6:821–833. [PubMed: 17906644]
- 45 •. Gridelli C, Rossi A, Bareschino MA, Schettino C, Sacco PC, Maione P. The potential role of insulin-like growth factor receptor inhibitors in the treatment of advanced non-small cell lung cancer. *Expert Opin. Investig. Drugs* 2010;19:631–639. Summarizes the potential of targeting IGF receptor in the treatment of non-small-cell lung cancer.
46. Yee D. Targeting insulin-like growth factor pathways. *Br. J. Cancer* 2006;94:465–468. [PubMed: 16450000]
47. Kulik G, Klippel A, Weber MJ. Antiapoptotic signaling by the insulin-like growth factor I receptor, phosphatidylinositol 3-kinase, and Akt. *Mol. Cell Biol* 1997;17:1595–1606. [PubMed: 9032287]
48. Pandini G, Frasca F, Mineo R, et al. Insulin/insulin-like growth factor I hybrid receptors have different biological characteristics depending on the insulin receptor isoform involved. *J. Biol. Chem* 2002;277(42):39684–39695. [PubMed: 12138094]
49. Denley A, Cosgrove LJ, Booker GW, et al. Molecular interactions of the IGF system. *Cytokine Growth Factor Rev* 2005;16:421–439. [PubMed: 15936977]

50. Chao W, D'Amore PA. IGF2: epigenetic regulation and role in development and disease. *Cytokine Growth Factor Rev* 2008;19:111–120. [PubMed: 18308616]
51. Spitz MR, Mistry J, et al. Plasma levels of insulin-like growth factor-I and lung cancer risk: a case-control analysis. *J. Natl Cancer Inst* 1999;91:151–156. [PubMed: 9923856]
52. Chang Y, Wang L, Liu D, et al. Correlation between insulin-like growth factor-binding protein-3 promoter methylation and prognosis of patients with stage I non-small cell lung cancer. *Clin. Cancer Res* 2002;8:3669–3675. [PubMed: 12473575]
53. Sordella R, Bell DW, Haber DA, Settleman J. Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. *Science* 2004;305:1163–1167. [PubMed: 15284455]
54. Cappuzzo F, Toschi L, Tallini G, et al. Insulin-like growth factor receptor 1 (IGFR-1) is significantly associated with longer survival in non-small cell lung cancer patients treated with gefitinib. *Ann. Oncol* 2006;17:1120–1127. [PubMed: 16600976]
55. Ludovini V, Bellezza G, Pistola L, et al. High coexpression of both insulin-like growth factor receptor-1 (IGFR-1) and epidermal growth factor receptor (EGFR) is associated with shorter disease-free survival in resected non-small cell lung cancer patients. *Ann. Oncol* 2009;20:842–849. [PubMed: 19153117]
56. Ullrich A, Gray A, Tam AW, et al. Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity. *EMBO J* 1986;5:2503–2512. [PubMed: 2877871]
57. Garber K. IGF-1: old growth factor shines as new drug target. *J. Natl Cancer Inst* 2005;97:790–792. [PubMed: 15928295]
58. Karp D, Pollak MN, Cohen RB, et al. Safety, pharmacokinetics, and pharmacodynamics of the insulin-like growth factor type 1 receptor inhibitor figitumumab (CP-751,871) in combination with paclitaxel and carboplatin. *J. Thorac. Oncol* 2009;4:1397–1403. [PubMed: 19745765]
59. Karp D, Paz-Ares LG, Novello S, et al. Phase II study of the anti-insulin-like growth factor type 1 receptor antibody CP-751,871 in combination with paclitaxel and carboplatin in previously untreated, locally advanced, or metastatic non-small cell lung cancer. *J. Clin. Oncol* 2009;27:2516–2522. [PubMed: 19380445]
60. Andrew S. PIK3CA: determining its role in cellular proliferation and ovarian cancer. *Clin. Genet* 1999;56:190–191. [PubMed: 10563477]
61. Osaki M, Oshimura M, Ito H. PI3K–Akt pathway: its functions and alterations in human cancer. *Apoptosis* 2004;9:667–676. [PubMed: 15505410]
62. Kandasamy K, Srivastava RK. Role of the phosphatidylinositol 3'-kinase/PTEN/Akt kinase pathway in tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis in non-small cell lung cancer cells. *Cancer Res* 2002;62:4929–4937. [PubMed: 12208743]
63. Brognard J, Clark AS, Ni Y, Dennis PA. Akt/protein kinase B is constitutively active in non-small cell lung cancer cells and promotes cellular survival and resistance to chemotherapy and radiation. *Cancer Res* 2001;61:3986–3997. [PubMed: 11358816]
64. Vignot S, Faivre S, Aguirre D, Raymond E. mTOR-targeted therapy of cancer with rapamycin derivatives. *Ann. Oncol* 2005;24:3348–3357.
65. Soria J, Shepherd FA, Douillard JY, et al. Efficacy of everolimus (RAD001) in patients with advanced NSCLC previously treated with chemotherapy alone or with chemotherapy and EGFR inhibitors. *Ann. Oncol* 2009;20:1674–1681. [PubMed: 19549709]
66. She Q, Solit DB, Ye Q, et al. The BAD protein integrates survival signaling by EGFR/MAPK and PI3K/Akt kinase pathways in PTEN-deficient tumor cells. *Cancer Cell* 2005;8:287–297. [PubMed: 16226704]
67. Engelman J, Mukokhara T, Zejnullahu K, et al. Allelic dilution obscures detection of a biologically significant resistance mutation in EGFR-amplified lung cancer. *J. Clin. Invest* 2006;116:2695–2706. [PubMed: 16906227]
68. Ferreira C, Epping M, Krzyt FA, Giaccone G. Apoptosis: target of cancer therapy. *Clin. Cancer Res* 2002;8:2024–2034. [PubMed: 12114400]
69. Duiker E, Mom CH, de Jong S, et al. The clinical trial of TRAIL. *Eur. J. Cancer* 2006;42:2233–2240. [PubMed: 16884904]

70. Ozoren N, El-Deiry WS. Defining characteristics of types I and II apoptotic cells in response to TRAIL. *Neoplasia* 2002;4:551–557. [PubMed: 12407450]
71. Kruyt F. TRAIL and cancer therapy. *Cancer Lett* 2008;263:14–25. [PubMed: 18329793]
72. Fang F, Wang AP, Yang SF. Antitumor activity of a novel recombinant mutant human tumor necrosis factor-related apoptosis-inducing ligand. *Acta Pharmacol. Sin* 2005;26:1373–1381. [PubMed: 16225761]
73. MacFarlane M, Kohlhaas SL, Sutcliffe MJ, Dyer MJ, Cohen GM. TRAIL receptor-selective mutants signal to apoptosis via TRAIL-R1 in primary lymphoid malignancies. *Cancer Res* 2005;65:11265–11270. [PubMed: 16357130]
74. Kelley R, Totpal K, Lindstrom SH, et al. Receptor-selective mutants of apoptosis-inducing ligand 2/tumor necrosis factor-related apoptosis-inducing ligand reveal a greater contribution of death receptor (DR) 5 than DR4 to apoptosis signaling. *J. Biol. Chem* 2005;280(3):2205–2212. [PubMed: 15520016]
75. Tur V, van der Sloot AM, Reis CR, et al. DR4-selective tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) variants obtained by structure-based design. *J. Biol. Chem* 2008;283:20560–20568. [PubMed: 18474604]
76. Yada A, Yazawa M, Ishida S, et al. A novel humanized anti-human death receptor 5 antibody CS-1008 induces apoptosis in tumor cells without toxicity in hepatocytes. *Ann. Oncol* 2008;19:1060–1067. [PubMed: 18304965]
77. Greco F, Bonomi P, Crawford J, et al. Phase 2 study of mapatumumab, a fully human agonistic monoclonal antibody which targets and activates the TRAIL receptor-1, in patients with advanced non-small cell lung cancer. *Lung Cancer* 2008;61:82–90. [PubMed: 18255187]
78. Luster T, Carrell JA, McCormick K, et al. Mapatumumab and lexatumumab induce apoptosis in TRAIL-R1 and TRAIL-R2 antibody-resistant NSCLC cell lines when treated in combination with bortezomib. *Mol. Cancer Ther* 2009;8:292–303. [PubMed: 19174554]
79. Jin H, Yang R, Ross J, et al. Cooperation of the agonistic DR5 antibody apomab with chemotherapy to inhibit orthotopic lung tumor growth and improve survival. *Cancer Res* 2008;64:4900–4905. [PubMed: 15256461]
80. Takahashi T, Nau MM, Chiba I, et al. P53: a frequent target for genetic abnormalities in lung cancer. *Science* 1989;246:491–494. [PubMed: 2554494]
81. Ferreira C, Span SW, Peters GH, Kruyt FA, Giaccone G. Chemotherapy triggers apoptosis in a caspase-8-dependent and mitochondria-controlled manner in the non-small cell lung cancer cell line NCI-H460. *Cancer Res* 2000;60:7133–7141. [PubMed: 11156422]
82. Spierings D, de Vries EG, Timens W, et al. Expression of TRAIL and TRAIL death receptors in stage III non-small cell lung cancer tumors. *Clin. Cancer Res* 2003;9:3397–3405. [PubMed: 12960128]
83. Frese S, Brunner T, Gugger M, Uduehi A, Schmid RA. Enhancement of Apo2L/ TRAIL (tumor necrosis factor-related apoptosis-inducing ligand)-induced apoptosis in non-small cell lung cancer cell lines by chemotherapeutic agents without correlation to the expression level of cellular protease caspase-8 inhibitory protein. *J. Thorac. Cardiovasc. Surg* 2002;123:168–174. [PubMed: 11782771]
84. Thorburn A, Behbakht K, Ford H. TRAIL receptor-targeted therapeutics: resistance mechanisms and strategies to avoid them. *Drug Resist. Updates* 2008;11:17–24.
85. Scagliotti G. Proteasome inhibitors in lung cancer. *Crit. Rev. Oncol. Hematol* 2006;58:177–189. [PubMed: 16427303]
86. Park S, Seol DW. Regulation of Akt by EGFR inhibitors, a possible mechanism of EGFR inhibitor-enhanced TRAIL-induced apoptosis. *Biochem. Biophys. Res. Commun* 2002;295:515–518. [PubMed: 12150980]
87. Soria J, Smit EF, Khayat D, et al. Phase Ib study of dulanermin (recombinant human Apo2L/ TRAIL) in combination with paclitaxel, carboplatin and bevacizumab in patients with advanced non-small cell lung cancer. *J. Clin. Oncol* 2008;28:1527–1533. [PubMed: 20159815]
88. Von Pawel J., Harvey, H.; Spigel, DR., et al. Randomized Phase II trial of mapatumumab, a TRAIL-R1 agonist monoclonal antibody, in combination with carboplatin and paclitaxel in

- patients with non-small-cell lung cancer (NSCLC); Presented at: 2010 Annual Oncology Meeting; Chicago, IL, USA. 4-8 June 2010; Abstract LBA 7501
89. LoRusso P, Hong D, Heath E, et al. First-in-human study of AMG 655, a pro-apoptotic TRAIL receptor-2 agonist, in adult patients with advanced solid tumors. *J. Clin. Oncol* 2007;25(Suppl. 18):3534. [PubMed: 17687158]
 90. Stegehuis J, de Wilt LHAM, de Vries EGE, Groen HJ, de Jong S, Kruijdt FAE. TRAIL receptor targeting therapies for non-small cell lung cancer: current status and perspectives. *Drug Resist. Updates* 2010;13:2–15. Describes the current view on the future potential of TNF-related apoptosis-inducing ligand-targeting therapies in non-small-cell lung cancer.
 91. Soo E, Yip GW, Lwin ZM, et al. Heat shock proteins as novel therapeutic targets in cancer. *In Vivo* 2008;22:311–315. [PubMed: 18610741]
 92. Donnelly A, Blagg BSJ. Novobiocin and additional inhibitors of the Hsp90 C-terminal nucleotide-binding pocket. *Curr. Med. Chem* 2008;15:2702–2717. [PubMed: 18991631]
 93. Mahalingam D, Swords R, Carew JS, et al. Targeting HSP90 for cancer therapy. *Br. J. Cancer* 2009;100:1523–1529. Good review article discussing the involvement of heat-shock protein (HSP)90 in cancer and the usefulness of targeted inhibition of HSP90 in cancer treatment. [PubMed: 19401686]
 94. Uehara Y. Natural product origins of Hsp90 inhibitors. *Curr. Cancer Drug Targets* 2003;3:325–330. [PubMed: 14529384]
 95. Conde R, Belak ZR, Nair M, et al. Modulation of Hsf1 activity by novobiocin and geldanamycin. *Biochem. Cell Biol* 2009;87:845–851. [PubMed: 19935870]
 96. Chandarlapaty S, Sawai A, Ye Q, et al. SNX2112, a synthetic heat shock protein 90 inhibitor, has potent antitumor activity against HER kinase-dependent cancers. *Clin. Cancer Res* 2008;14:240–248. [PubMed: 18172276]
 97. Radons J, Multhoff G. Immunostimulatory functions of membrane-bound and exported heat shock protein 70. *Exerc. Immunol. Rev* 2005;11:17–33. [PubMed: 16385841]
 98. Kallio P, Pongratz I, Gradin K, McGuire J, Poellinger L. Activation of hypoxia-inducible factor 1 α : posttranscriptional regulation and conformational change by recruitment of the Arnt transcription factor. *Proc. Natl Acad. Sci. USA* 1997;94:5667–5672. [PubMed: 9159130]
 99. Semenza G. Hypoxia-inducible factor 1: master regulator of O₂ homeostasis. *Curr. Opin. Genet. Dev* 1998;8:588–594. [PubMed: 9794818]
 100. Elson D, Thurston G, Huang LE, et al. Induction of hypervascularity without leakage or inflammation in transgenic mice overexpressing hypoxia-inducible factor-1 α . *Genes Dev* 2001;15:2520–2532. [PubMed: 11581158]
 101. Brahimi-Horn M, Pouyssegur J. The hypoxia-inducible factor and tumor progression along the angiogenic pathway. *Int. Rev. Cytol* 2005;242:157–213. [PubMed: 15598469]
 102. Jones P, Baylin SB. The epigenomics of cancer. *Cell* 2007;128:683–692. [PubMed: 17320506]
 103. Luger K. Structure and dynamic behavior of nucleosomes. *Curr. Opin. Genet. Dev* 2003;13:127–135. [PubMed: 12672489]
 104. Lane A, Chabner BA. Histone deacetylase inhibitors in cancer therapy. *J. Clin. Oncol* 2009;27:5459–5468. Reviews the important roles of epigenetic alterations in human cancer and the potential of inhibiting histone deacetylase as a therapeutic regimen. [PubMed: 19826124]
 105. Strahl B, Allis CD. The language of covalent histone modifications. *Nature* 2000;403:41–45. [PubMed: 10638745]
 106. Gregory P, Wagner K, Horz W. Histone acetylation and chromatin remodeling. *Exp. Cell Res* 2001;265:195–202. [PubMed: 11302684]
 107. Roth S, Denu JM, Allis CD. Histone acetyltransferases. *Annu. Rev. Biochem* 2001;70:81–120. [PubMed: 11395403]
 108. Gray S, Ekstrom TJ. The human histone deacetylase family. *Exp. Cell Res* 2001;262:75–83. [PubMed: 11139331]
 109. Zhang Y, Adachi M, Kawamura R, et al. Bmf is a possible mediator in histone deacetylase inhibitors FK228 and CBHA-induced apoptosis. *Cell Death Differ* 2006;13:129–140. [PubMed: 15947789]

110. Zhao Y, Tan J, Zhuang L, et al. Inhibitors of histone deacetylases target the Rb-E2F1 pathway for apoptosis induction through activation of proapoptotic protein Bim. *Proc. Natl Acad. Sci. USA* 2005;102:16090–16095. [PubMed: 16243973]
111. Xu Y. Regulation of p53 responses by post-translational modifications. *Cell Death Differ* 2003;10:400–403. [PubMed: 12719715]
112. Kim M, Kwon HJ, Lee YM, et al. Histone deacetylases induce angiogenesis by negative regulation of tumor suppressor genes. *Nat. Med* 2001;7:437–543. [PubMed: 11283670]
113. Fath D, Kong X, Liang D, et al. Histone deacetylase inhibitors repress the transactivation potential of hypoxia-inducible factors independently of direct acetylation of HIF- α . *J. Biol. Chem* 2006;281:13612–13619. [PubMed: 16543236]
114. Geng L, Cuneo KC, Fu A, et al. Histone deacetylase (HDAC) inhibitor LBH589 increases duration of g-H2AX foci and confines HDAC4 to the cytoplasm in irradiated non-small cell lung cancer. *Cancer Res* 2006;66:11298–11304. [PubMed: 17145876]
115. Edwards A, Li J, Atadja P, et al. Effect of the histone deacetylase inhibitor LBH589 against epidermal growth factor receptor-dependent human lung cancer cells. *Mol. Cancer Ther* 2007;6:2515–2524. [PubMed: 17876048]
116. Yagui-Beltrán A, He B, Raz D, Kim J, Jablons DM. Novel therapies targeting signaling pathways in lung cancer. *Thorac. Surg. Clin* 2006;16:379–396. [PubMed: 17240825]
117. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat. Med* 1997;3:730–737. [PubMed: 9212098]
118. O'Brien C, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* 2007;445:106–110. [PubMed: 17122772]
119. Ricci-Vitiani L, Lombardi DG, Pilozzi E, et al. Identification and expansion of human colon-cancer-initiating cells. *Nature* 2007;445:111–115. [PubMed: 17122771]
120. Widelitz R. Wnt signaling through canonical and non-canonical pathways: recent progress. *Growth Factors* 2005;23:111–116. [PubMed: 16019432]
121. Akiyama T. Wnt/ β -catenin signaling. *Cytokine Growth Factor Rev* 2000;11:273–282. [PubMed: 10959075]
122. He B, You L, Uematsu K, et al. A monoclonal antibody against Wnt-1 induces apoptosis in human cancer cells. *Neoplasia* 2004;6:7–14. [PubMed: 15068666]
123. Chen S, Guttridge DC, You Z. Wnt-1 signaling inhibits apoptosis by activating β -catenin/T cell factor-mediated transcription. *J. Cell Biol* 2001;152:87–96. [PubMed: 11149923]
124. Uematsu K, He B, You L, et al. Activation of the Wnt pathway in non small cell lung cancer: evidence of disheveled overexpression and transcriptional activity of β -catenin. *Oncogene* 2003;22:7218–7221. [PubMed: 14562050]
125. Kim J, You L, Xu Z, et al. Wnt inhibitory factor inhibits lung cancer cell growth. *J. Thorac. Cardiovasc. Surg* 2007;133:733–737. [PubMed: 17320573]
126. He B, Barg RN, You L, et al. Wnt signaling in stem cells and non-small-cell lung cancer. *Clin. Lung Cancer* 2005;7:54–60. [PubMed: 16098245]
127. Lepourcelet M, Chen YN, France DS, et al. Small-molecule antagonists of the oncogenic Tcf/ β -catenin protein complex. *Cancer Cell* 2004;5:91–102. [PubMed: 14749129]
128. Emami K, Nguyen C, Ma H. A small molecule inhibitor of β -catenin/CREB-binding protein transcription. *Proc. Natl Acad. Sci. USA* 2004;101:12682–12687. [PubMed: 15314234]
129. Barker N, Clevers H. Mining the Wnt pathway for cancer therapeutics. *Nat. Rev. Drug Discov* 2006;5:997–1014. [PubMed: 17139285]
130. Piyathilake C, Frost AR, Manne U, et al. Differential expression of growth factors in squamous cell carcinoma and precancerous lesions of the lung. *Clin. Cancer Res* 2002;8:734–744. [PubMed: 11895903]
131. Lonardo F, Dragnev KH, Freemantle SJ, et al. Evidence for the epidermal growth factor receptor as a target for lung cancer prevention. *Clin. Cancer Res* 2002;8:54–60. [PubMed: 11801540]
132. Volm M, Koomagi R, Mattern J. Prognostic value of p16INK4A expression in lung adenocarcinoma. *Anticancer Res* 1998;18:2309–2312. [PubMed: 9703871]

133. Hall A, Dix BR, O'Carroll SJ, et al. p53 status does not determine outcome of E1B 55-kilodalton mutant adenovirus lytic infection. *J. Clin. Oncol* 1998;21:4546–4552.
134. Von Hoff D, LoRusso PM, Rudin CM, et al. Inhibition of the hedgehog pathway in advanced basal-cell carcinoma. *N. Engl. J. Med* 2009;361:1164–1172. The first article to demonstrate that a stem-cell pathway inhibitor shows promising clinical efficacy in cancer patients. [PubMed: 19726763]
135. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming *EML4-ALK* fusion gene in non-small-cell lung cancer. *Nature* 2007;448:561–566. [PubMed: 17625570]
136. Shaw A, Yeap BY, Mino-Kenudson M, et al. Clinical features and outcome of patients with non-small-cell lung cancer who harbor *EML4-ALK*. *J. Clin. Oncol* 2009;27:4247–4253. [PubMed: 19667264]
137. Inamura K, Takeuchi K, Togashi Y, et al. *EML4-ALK* lung cancers are characterized by rare other mutations, a TTF-1 cell lineage, an acinar histology, and young onset. *Mod. Pathol* 2009;22:508–515. [PubMed: 19234440]
138. Kwak E, Camidge DR, Clark J, et al. Clinical activity observed in a Phase I dose escalation trial of an oral c-met and *ALK* inhibitor, PF-02341066. *J. Clin. Oncol* 2009;27(15S) abstract 3509.
139. Bang, Y.; Kwak, E.; Shaw, AT., et al. Clinical activity of the oral *ALK* inhibitor PF-0234106 in *ALK*-positive patients with non-small-cell lung cancer; Presented at: 2010 Annual Oncology Meeting; Chicago, IL, USA. 4-8 June 2010; Abstract 3
140. Cronin M, Sangli C, Liu ML, et al. Analytical validation of the Oncotype DX genomic diagnostic test for recurrence prognosis and therapeutic response prediction in node-negative, estrogen receptor-positive breast cancer. *Clin. Chem* 2007;53:1084–1091. [PubMed: 17463177]
141. Raz D, Ray MR, Kim JY, et al. A multigene assay is prognostic of survival in patients with early-stage lung adenocarcinoma. *Clin. Cancer Res* 2008;14:5565–5570. [PubMed: 18765549]
142. Chen H, Yu SL, Chen CH, et al. A five-gene signature and clinical outcome in non-small-cell lung cancer. *N. Engl. J. Med* 2007;356:11–20. [PubMed: 17202451]
143. Skrzypski M, Jassem E, Taron M, et al. Three-gene expression signature predicts survival in early-stage squamous cell carcinoma of the lung. *Clin. Cancer Res* 2008;14:4794–4799. [PubMed: 18676750]
144. Kim IJ, Kim K, Kang HC, Jang SG, Park JG. DHPLC analysis of adenomatous polyposis coli (*APC*) mutations using ready-to-use *APC* plates: simple detection of multiple base pair deletion mutations. *Genet. Test* 2008;12(2):295–298. [PubMed: 18554166]

Patents

201. Blaschuk, OW.; Byers, S.; Gour, BJ. Compounds and methods for modulating β -catenin mediated gene expression. US6303576. 2001.
202. Moll, J.; Knapp, S.; Dalvit, C.; Trosset, JY.; Sundstrom, M.; Mantegani, S. Interaction inhibitors of TCF-4 with β -catenin. EP-1406889. 2004.
203. Kahn, M.; Eguch, M.; Moon, SH.; Chung, JU.; Jeong, KW. Compounds useful for treatment of cancer, compositions containing the same, and methods of their use. US6762185. 2004.

Websites

301. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology (TM): non-small cell lung cancer V.1.\20. 2009. www.nccn.org/professionals/physician_gls/PDF/nscl.pdf (Accessed May 12 2010)
302. Clinicaltrials.gov. www.clinicaltrials.gov (Accessed July 30 2010)
303. ATTRACT-1 study. www.attractstudy.com/attract-1-researching-ASA404-in-cancer-treatment.jsp (Accessed July 30 2010)
304. Pfizer Oncology: Pfizer discontinues a Phase 3 study of figitumumab in previously treated patients with advanced non-small cell lung cancer.

http://media.pfizer.com/files/news/press_releases/2010/figitumumab_031110.pdf (Accessed May 18 2010)

305. Pfizer Press Release Archive.

www.pfizer.com/news/press_releases/pfizer_press_release_archive.jsp (Accessed May 18 2010)

Figure 1. Key signaling pathways for targeted therapies in lung cancer

DVL: Disheveled; EGFR: EGF receptor; FADD: Fas-associated death domain; FZ: Frizzled; GLI: Glioma-associated oncogene homolog; HDAC: Histone deacetylase; HIF: Hypoxia-inducible factor; IGF-1R: IGF receptor; LEF: Lymphoid enhancer factor; PTCH: Patched; SHH: Sonic hedgehog; SMO: Smoothed; TCF: T-cell factor; TRAIL: TNF-related apoptosis-inducing ligand; VEGFR: VEGF receptor; WNT: Wingless-int proto-oncogene.

Table 1

Examples of key clinical trials in lung cancer.

Pathway	Drug name	Target	Lung cancer type	Phase
EGFR	HKI-272	EGFR, HER2	NSCLC, stage IIIB/IV	II (completed)
	XL647	EGFR, HER2, VEGFR	Lung adenocarcinoma, stage IIIB/IV	II (completed)
	BIBW2992	EGFR (wild-type and mutant), HER2	Lung adenocarcinoma, stage IIIB/IV	III
	PF299804	EGFR, HER2, HER4		II (completed) [†]
VEGF	Vandetanib	VEGFR-2, EGFR	Refractory NSCLC	III
			Refractory NSCLC	III
	Sunitinib	VEGFR-1, VEGFR-2, FLT3, PDGFR- α , PDGFR- β	NSCLC, stage IIIB/IV	III
	Sorafenib	VEGFR-1, VEGFR-2, FLT3, PDGFR- α , PDGFR- β	NSCLC, stage IIIB/IV	III (completed)
	BIBF1120	VEGFRs, PDGFRs, FGFRs	NSCLC	III
			NSCLC	III
	Motesanib	All known VEGFRs, PDGFR, Kit	Unresectable, nonsquamous NSCLC, stage IIIB/IV	III
	AZD2171	All known VEGFRs	Small-cell lung cancer	II (completed)
			NSCLC, stage IIIB/IV	III
VEGF + EGFR	Bevacizumab + erlotinib	VEGFA, EGFR	NSCLC	III
			BAC, stage IIIB/IV	II
			Nonsmokers with lung adenocarcinoma, stage IIIB/IV	III
IGF	Figitumumab	IGF-1R	Large cell, squamous or adenosquamous lung carcinomas	III (terminated)
			Small-cell lung cancer	II
	R1507	IGF-1R	NSCLC, stage IIIB/IV	II
	MK0646	IGF-1R	NSCLC, stage IIIB/IV	II
	AMG479	IGF-1R	Squamous-cell lung carcinoma, stage IIIB/IV	I/II
	Cixutumumab	IGF-1R	NSCLC, stage III/IV Nonsquamous NSCLC, stage IV	II II
PI3K/Akt	GDC-0941	PI3K	NSCLC, stage IIIB/IV	I
	Rapamycin	mTOR	Nonsquamous NSCLC	I
			NSCLC	I/II
	Everolimus	mTOR	NSCLC, stage IIIB/IV Small-cell lung cancer	II (completed) II
TRAIL	AMG951	Apo2L/TRAIL	NSCLC, stage IIIB/IV	II
	Mapatumumab	TRAIL-R1	NSCLC, stage IIIB/IV	II (completed)
	AMG655	TRAIL-R2	NSCLC	I/II

Pathway	Drug name	Target	Lung cancer type	Phase
HSP	IPI-504	HSP90	NSCLC, stage IIIB/IV	I/II
			ALK mutation and NSCLC, stage IIIB/IV	II
	STA-9090	HSP90	NSCLC, stage IIIB/IV	II
HDAC	CI-994	Histone deacetylation	NSCLC, stage IIIB/IV	III
	Vorinostat	HDAC1,2, HDAC3 (class I), HDAC6 (class II)	NSCLC, stage IIIB/IV	III (terminated)
			EGFR mutation and NSCLC, stage IIIB/IV	I/II
	Panobinostat	Histone deacetylation	NSCLC, stage IIIB/IV	I
			Untreated small-cell lung cancer	I
EML4/ALK	Crizotinib	ALK	ALK-mutation-positive NSCLC	III

[†]Data from reference [21].

ALK: Anaplastic lymphoma kinase; Apo2L: Apo2 ligand; BAC: Bronchoalveolar carcinoma; EGFR: EGF receptor; EML: Echinoderm microtubule-associated protein-like; FGFR: FGF receptor; FLT: FMS-like tyrosine kinase; HDAC: Histone deacetylase; HER: Human epidermal growth factor receptor; HSP: Heat-shock protein; IGF-1R: IGF receptor; NSCLC: Non-small-cell lung cancer; PDGFR: PDGF receptor; TRAIL: TNF-related apoptosis-inducing ligand; VEGFR: VEGF receptor.

More detailed information on these and other trials can be found at [304].