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From Biology to Therapy: Improvements of Therapeutic Options in Lung Cancer

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Abstract

Lung cancer is the leading cause of cancer-related mortality around the world, despite effective chemotherapeutic agents, the prognosis has remained poor for a long time. The discovery of molecular changes that drive lung cancer has led to a dramatic shift in the therapeutic landscape of this disease. In “*in vitro*” and “*in vivo*” models of NSCLC (non-small cell lung cancer), angiogenesis blockade has demonstrated an excellent anti-tumor activity, thus, a number of anti-angiogenic drugs have been approved by regulatory authorities for use in clinical practice. Much more interesting is the discovery of EGFR (epithelial growth factor receptor) mutations that predict sensitivity to the anti-EGFR tyrosine kinase inhibitors (TKIs), a class of drugs that has shown to significantly improve survival when compared with standard chemotherapy in the first-line treatment of metastatic NSCLC. Nevertheless, after an initial response, resistance often occurs and prognosis becomes dismal. Biomolecular studies on cell line models have led to the discovery of mutations (*e.g.*, T790M) that confer resistance to anti-EGFR inhibitors. Fortunately, drugs that are able to circumvent this mechanism of resistance have been developed and have been recently approved for clinical use. The discovery of robust intra-tumor lymphocyte infiltration in NSCLC has paved the way to several strategies able to restore the immune response. Thus, agents interfering with PD-1/PD-L1 (programmed death) pathways make up a significant portion of the armamentarium of cancer therapies for NSCLC. In all the above-mentioned situations, the basis of the success in treating NSCLC has started from understanding of the mutational landscape of the tumor.

While lung cancer remains the leading cause of cancer-related mortality around the world [1], many therapeutic advances have been made in recent years to improve on the survival of patients with advanced non-small cell lung cancer (NSCLC) and exemplifies the bench-to-bedside model of translational research [2]. Herein, we briefly describe the advances of key molecularly targeted agents in NSCLC.

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CONFLICT OF INTEREST

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CONSENT FOR PUBLICATION

Not applicable.

Keywords

Cancer; EGFR mutations; cancer related mortality; lung cancer; therapeutics; non-small cell lung cancer

ANTIANGIOGENIC AGENTS

The study of tumor angiogenesis started a long time ago [3]. The vascular endothelial growth factor (VEGF)/VEGF-receptor (VEGFR) pathway plays a pivotal role in normal and pathological angiogenesis [4]. VEGF overexpression has been showed in most human cancers, including NSCLC and is associated with increased tumor recurrence, metastasis and death [5]. The VEGF/VEGFR pathway is involved in different physiological and pathological processes. The activation of this pathway is necessary for endothelial cell survival, mitogenesis, migration, differentiation and mobilization of endothelial progenitor cells from the bone marrow into the peripheral circulation [4, 5]. The pathway is composed of six secreted glycoproteins referred to as VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placenta growth factor (PlGF) –1 and –2 [6–8] that mediate their effect through several receptor tyrosine kinases [4, 5]. Most isoforms of VEGF bind to VEGFR-1 and VEGFR-2, whereas PlGF-1 and –2 and VEGF-B specifically bind and activate VEGFR-1 [9–11]. The principal physiological and pathological effects such as microvascular permeability, migration or invasion are mediated by binding of VEGFR-2 with the VEGF ligands [12–14]. Based on these central processes in angiogenesis, different strategies have been developed in recent years to target the VEGF/VEGFR pathway including neutralizing antibodies to VEGF (bevacizumab and aflibercept) or VEGFR-2 (ramucirumab) and receptor tyrosine kinase inhibitors (TKIs) with preferential selectivity for the VEGFRs, such as nintedanib. In 2006, bevacizumab was approved for the treatment of unresectable, locally advanced, recurrent, or metastatic NSCLC [5]. Preclinical results highlighted the capability of bevacizumab to inhibit the growth of various human tumor cell types in *in vivo* but not *in vitro* models, including the CALU-6 NSCLC model [15–17]. The analysis of the treated tumors showed reduced vascularity [17] and reduced interstitial pressure [16, 18]. Other studies found that these drugs were also able to reduce metastasis formation [19, 20]. The production of VEGF induced by hypoxia is a mechanism of tumor resistance to radiotherapy and chemotherapy [21], and bevacizumab significantly augments the antitumor effects of both modalities [15]. Two large randomized phase III trials, the Intergroup trials E4599 and the Avastin in Lung study (AVAL), evaluated the addition of bevacizumab to platinum-based doublet chemotherapy in patients with advanced non-squamous NSCLC in the first-line setting. E4599 trial showed that the addition of bevacizumab to carboplatin/paclitaxel significantly improved median overall survival (OS) in patients with advanced non-squamous NSCLC compared to chemotherapy alone (12.3 vs 10.3 months, hazard ratio (HR) 0.79, $p=0.003$). The addition of bevacizumab was associated with an increase in clinically significant bleeding and hematologic toxicity compared with carboplatin/paclitaxel alone [22]. The AVAL study compared three different treatment regimens: 7.5 mg/kg bevacizumab plus cisplatin/gemcitabine or 15 mg/kg bevacizumab plus cisplatin/gemcitabine vs. standard chemotherapy in patients with advanced non squamous NSCLC. This trial showed a significant prolongation in progression free survival (PFS) from 6.1 to

6.7 months in the group treated with the 7.5 mg/kg dose of bevacizumab (HR 0.75, $p=0.003$) and 6.1 to 6.5 months in the group treated with the 15 mg/kg dose of bevacizumab (HR 0.82, $p=0.03$) as compared to standard chemotherapy [23].

Unlike bevacizumab, ramucirumab binds to VEGFR-2 and blocks the binding of VEGFR-ligands (VEGF-A, VEGF-C, and VEGF-D) to the receptor [24]. Ramucirumab has a higher binding affinity to the extracellular portion of VEGFR-2 as compared to the natural VEGF-ligands [25]. Based on the results of REVEL trial, ramucirumab is approved for the second line treatment of metastatic NSCLC in combination with docetaxel [26]. In this Phase III, multicenter, randomized, double-blind study, the combination of ramucirumab plus docetaxel was compared to docetaxel plus placebo in patients with stage IV NSCLC. This clinical trial showed a marginally (5 weeks) but significant improvement of OS.

Nintedanib, a multi-TKI active against VEGFR-1, VEGFR-2, VEGFR-3, FGFR-1, 2, 3, PDGFR- α and β , FLT3, and SRC family members [27, 28], is approved in combination with chemotherapy as a second line treatment for lung cancer patients with advanced adenocarcinoma. In preclinical studies, nintedanib combined with chemotherapy was able to arrest the growth and increased apoptosis of NSCLC tumors xenografts resistant to bevacizumab and platinum-doublet chemotherapy [29]. In the multicenter, randomized, double-blind, phase III LUME-Lung 1 trial, in patients with stage IIIB/IV NSCLC progressing after first-line chemotherapy, the combination of nintedanib plus docetaxel showed a manageable safety profile and an increase in OS compared to docetaxel alone [30]. These results led the approval of nintedanib in Europe in 2014.

In addition to the VEGF/VEGFR pathway, others important factors have also been shown to affect tumor-associated pathophysiologic-relevant processes such as angiogenesis, growth, metastasis, migration and thrombogenic potential. One such factor is the expression of tissue factor (TF) in NSCLC [31]. TF consists of a 47-kDa transmembrane protein and is best as an initiator of the extrinsic coagulation cascade [32, 33]. A soluble alternatively spliced isoform of TF (asTF) was identified more than 10 years ago. asTF is not required for normal hemostasis, however, both isoform expression level positively correlates with advanced tumor stages in several human cancers including cervical cancer [34], epithelial ovarian cancer [35], breast cancer [36], brain tumor [37], pancreatic cancer [38] and lung cancer [39]. Moreover, asTF has been shown to drive proliferation and angiogenesis in lung cancer cells through integrin signaling. In fact, direct binding between asTF and integrins β 3 and β 1 induces angiogenesis *in vitro* and *in vivo* experiments [40]. As described by Eisenreich and colleagues, targeting flTF and asTF may offer novel potential options for the treatment of these cancers [41]. Strategies targeting TF and its isoforms have shown promising preclinical results, however, additional clinical and translational studies are needed to fully assess the benefit in patients.

TYROSINE-KINASE INHIBITORS

Activation of the receptor tyrosine kinases (RTKs) is fundamental in the regulation of cellular growth and survival, organ morphogenesis, neovascularization, and tissue repair and regeneration. Many human cancers display a dysregulated or constitutive activation of

RTKs. This deregulation can be mediated by different mechanisms such as gain-of-function mutations, gene rearrangements, gene amplification, over-expression or abnormal autocrine, endocrine or paracrine stimulation of both receptors and ligands. RTK over-activation has often been shown to correlate with the development and progression of numerous human cancers [42] (REF!!!). Since RTKs have been implicated in many aspects of the malignant phenotype, they have emerged as promising therapeutic targets. Indeed, different receptors have already been identified as targets to cure lung cancers [43]. The epidermal growth factor receptor (EGFR) is a crucial element for the development and growth of human malignancies, including lung cancer [44]. Somatic mutations in the EGFR gene in NSCLC has led to the identification of a biologically distinct disease entity so-called 'oncogene addicted' to reflect its dependence on EGFR-mediated pro-survival signaling [45–48]. EGFR mutations in NSCLC are spatially located within the ATP-binding site of the kinase. The most frequent EGFR mutations are in-frame deletions (amino acid residues between 747 to 750) of exon 19 (45%); the exon 21 L858R mutations (40%); and in-frame insertions within exon 20 (5–10%) of the kinase [49, 50]. EGFR mutations are more frequent in tumors with adenocarcinoma histology, in never smokers or light smokers, in women with NSCLC, and in patients with East Asian ethnicities [51]. The mechanism of activation and response to TKIs such as erlotinib, gefitinib and afatinib has been explained by the crystal structures and enzymatic assays of several EGFR mutations (*e.g.*, L858R, G719S, T790M, and D770_N771insNPG) [52]. These mutations increase the activation state of the receptor by enhancing mutant EGFR homodimerization and heterodimerization with wild-type (WT) EGFR [52–55]. In addition, these mutations decrease the affinity for ATP and increased the affinity for EGFR TKIs compared to the WT receptor [53]. Hence, the presence of EGFR mutations represents a therapeutic window for EGFR TKIs. In fact, in preclinical models, nanomolar concentration of gefitinib, erlotinib, and afatinib can inhibit classic EGFR mutants while sparing the WT receptors. EGFR mutations, by favoring the active kinase state, are oncogenic in NSCLC cell lines, mouse models, and human tumors. These effects are mediated through the activation of anti-apoptotic and prosurvival proteins *via* downstream targets of the mitogen-activated protein kinase/extracellular-signal-regulated kinase (MAPK/ERK), janus kinase/signal transducer and activator of transcription (JAK/STAT), and phosphatidylinositol-3-kinase/protein kinase B cascades (PI3K/PKB) [56]. The inhibition of EGFR mutant leads to upregulation and activation of pro-apoptotic molecules that initiate the intrinsic mitochondrial apoptotic pathway by affecting the balance of pro- vs anti-apoptotic BCL-2 (B-cell lymphoma 2) family members [57]. Before the identification of the role of EGFR mutation as a predictive biomarker of sensitivity to the EGFR TKIs, two oral reversible EGFR TKIs, gefitinib and erlotinib, were approved for the treatment of unselected NSCLC with a minimal clinical benefit [58, 59]. Later randomized studies conducted in selected patient populations confirmed the predictive value of EGFR mutations as the major biomarker for the beneficial effects of gefitinib [60, 61], erlotinib [62], and afatinib [63]. Studies comparing EGFR TKIs versus systemic platinum-based chemotherapy as first-line treatment of advanced NSCLC, showed improved response rate (RR), longer PFS and better quality of life with EGFR TKI compared platinum-doublets [60, 62–69]. The main limitation of such inhibitors is the development of acquired resistance. The first identified, and most common, mechanism of acquired resistance is acquisition of the EGFR-T790M point mutation located at a key position in the ATP-binding cleft which is capable of

abolishing the sensitization of the activating mutation [53, 70]. Third generation of the covalent pyrimidine inhibitors of EGFR-T790M, such as osimertinib [71] or rociletinib [72], has been developed. These drugs are more potent against EGFR-T790M and less potent against WT EGFR than erlotinib, gefitinib and afatinib [72–74]. A randomized, international, open-label, phase III trial showed that osimertinib increases PFS when compared with chemotherapy (10.1 vs 4.4; HR, 0.30; 95% CI, 0.23–0.41; $p < 0.01$) in patients with EGFR-T790M metastatic NSCLC [75]. This led to Food and Drug Administration (FDA) approval of osimertinib for patients with metastatic EGFR T790M-positive NSCLC who have progressed on or after EGFR TKI therapy.

Beside EGFR mutations, other targetable molecular alterations have been described in lung cancer. According to recent studies gene rearrangements between the anaplastic lymphoma kinase (ALK) and the echinoderm microtubule-associated protein like-4 (EML4) are detected in 3–5% of NSCLCs [76, 77]. EML4-ALK translocation generates a transforming tyrosine kinase [78] and represents a novel molecular target in a small subset of NSCLCs. Patients with EML4-ALK positive tumors are usually of younger age, female, and never to light smokers [79, 80]. Multiple variants of EML4-ALK have been reported, all of which encode the same cytoplasmic portion of ALK but contain different truncations of EML4 [78]. The EML4 fusion partner mediates ligand-independent dimerization and/or oligomerization of ALK, resulting in constitutive kinase activity. The EML4-ALK translocation demonstrated potent oncogenic activity in cell culture system [78] and led to the development of lung adenocarcinoma in transgenic mice [81]. On these bases, the FDA has approved different TKI that target ALK - crizotinib, ceritinib, brigatinib and alectinib. In a phase III randomized clinical study in patients with locally advanced or metastatic NSCLC who were positive for ALK gene rearrangement, treatment with crizotinib versus chemotherapy improved PFS, quality of life, and response rates [82]. The FDA approved ceritinib and alectinib for patients with ALK-positive metastatic NSCLC who have progressed on are intolerant to crizotinib [83, 84].

Another rare alteration in NSCLC is the presence of protooncogene tyrosine-protein kinase ROS (ROS1) gene-rearrangements that occur in about 1% of patients with NSCLC. ALK and ROS1 rearrangements rarely arise in the same tumor, each defining a unique molecular subgroup of NSCLC [85]. Crizotinib has been shown to be effective in patients with ROS1 translocation with response rates of about 70% including complete responses [86].

IMMUNOTHERAPY

For a long time, lung cancer was considered to be nonimmunogenic. Recently, due in part to the success of immunotherapy in malignant melanoma, there has been great interest and investigation in the immune checkpoint inhibitors for the treatment of NSCLC. The goal of immunotherapy is to recognize and eradicate cancer cells by restoring the capacity of the host's immune. The capability of some cancer cells to escape the immune system is due to their ability to inhibit the effector T-cells. One of the best-known mechanisms of immune-escape is the expression of programmed cell death ligand 1 (PD-L1) by tumor cells [87]. PD-L1 is expressed in about 60% of NSLCs and can bind to the cluster of differentiation 8 (CD8) T-cell PD-1 receptor, resulting in the inhibition of the effector T-cell and evasion from

the immune system [88]. Currently, approved PD-1 targeted agents include nivolumab and pembrolizumab which are humanized IgG4 monoclonal antibodies (mAb) against PD-1 and atezolimumab which is an IgG1 anti-PD-L1 mAb designed to prevent antibody-dependent T-cell-mediated cytotoxicity (ADCC). In two different phase III trial, CheckMate 017 [89] and CheckMate 057 [90] nivolumab showed promising results as a second line treatment compared to docetaxel in patients who progressed after a platinum-containing regimen. CheckMate 017 enrolled patients with stage IIIB or IV squamous NSCLC, while CheckMate 057 evaluated the role of this drug in nonsquamous NSCLC. In both studies, nivolumab improved OS and PFS rates. Therefore, nivolumab received a non-restricted FDA approval as a second line therapy for metastatic NSCLC with progression on or after standard therapy. The KEYNOTE-024 trial, comparing pembrolizumab versus investigator's choice of chemotherapy as first-line of treatment for patients with advanced NSCLC and 50% tumor score for PD-L1, showed a longer PFS (10.3 vs 6 months, $p<0.001$) and OS at 6 months (80.2% vs 72.4%, $p=0.005$) in the arm treated with immunotherapy [91]. Atezolimumab (anti-PD-L1) was approved at the end of 2016 for the treatment of patients with PD-L1 positive NSCLC whose cancer has progressed during or after standard treatment based on the phase II POPLAR trial [92] and the phase III OAK trial [93] showing increased OS in the atezolimumab group versus docetaxel.

CONCLUSION

The era of genomic medicine particularly in oncology has paved the way for targeted therapies aimed at stopping the so-called “driver-mutations” and hallmarks of cancer. The first targets were aimed at blocking angiogenesis and RTKs. These drugs are highly effective in a select population of patients and have changed the landscape of NSCLC treatment (for example erlotinib in EGFR mutant NSCLC) sparing patients from cytotoxic chemotherapy. More recently, immunotherapy has emerged as the front-runner in the fight against NSCLC leading to the rapid approval of several agents. NSCLCs are a very heterogeneous group of malignancies that should be divided on the basis of their mutational landscape; many of them carry driver mutations in key genes (*e.g.*, EGFR, ALK, ROS1, MET) for which targeted therapies exist while others are very sensitive to angiogenesis blockade. Finally, the impact of the immune response on the neoplastic progression of NSCLC has further complicated the treatment landscape; however, biomarkers such as PD-L1 expression can potentially select which NSCLC tumors will respond to immunotherapy.

REFERENCES

- [1]. Zhang H Apatinib for molecular targeted therapy in tumor. *Drug Des Devel Ther.* 2015; 9: 6075–81.
- [2]. Goldblatt EM, Lee WH. From bench to bedside: the growing use of translational research in cancer medicine. *Am J Transl Res.* 2010; 2(1): 1–18. [PubMed: 20182579]
- [3]. Folkman J Tumor angiogenesis: therapeutic implications. *N Engl J Med.* 1971; 285(21): 1182–6. [PubMed: 4938153]
- [4]. Hicklin DJ, Ellis LM. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. *J Clin Oncol.* 2005; 23(5): 1011–27. [PubMed: 15585754]
- [5]. Villaruz LC, Socinski MA. The role of anti-angiogenesis in non-small-cell lung cancer: an update. *Curr Oncol Rep.* 2015; 17(6): 26. [PubMed: 25947099]

- [6]. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med.* 2003; 9(6): 669–76. [PubMed: 12778165]
- [7]. Houck KA, Ferrara N, Winer J, Cachianes G, Li B, Leung DW. The vascular endothelial growth factor family: identification of a fourth molecular species and characterization of alternative splicing of RNA. *Mol Endocrinol.* 1991; 5(12): 1806–14. [PubMed: 1791831]
- [8]. Tischer E, Mitchell R, Hartman T, Silva M, Gospodarowicz D, Fiddes JC, et al. The human gene for vascular endothelial growth factor. Multiple protein forms are encoded through alternative exon splicing. *J Biol Chem.* 1991; 266(18): 11947–54. [PubMed: 1711045]
- [9]. Park JE, Chen HH, Winer J, Houck KA, Ferrara N. Placenta growth factor. Potentiation of vascular endothelial growth factor bioactivity, *in vitro* and *in vivo*, and high affinity binding to Flt-1 but not to Flk-1/KDR. *J Biol Chem.* 1994; 269(41): 25646–54. [PubMed: 7929268]
- [10]. Olofsson B, Korpelainen E, Pepper MS, Mandriota SJ, Aase K, Kumar V, et al. Vascular endothelial growth factor B (VEGF-B) binds to VEGF receptor-1 and regulates plasminogen activator activity in endothelial cells. *Proc Natl Acad Sci U S A.* 1998; 95(20): 11709–14. [PubMed: 9751730]
- [11]. Silvestre JS, Tamarat R, Ebrahimian TG, Le-Roux A, Clergue M, Emmanuel F, et al. Vascular endothelial growth factor-B promotes *in vivo* angiogenesis. *Circ Res.* 2003; 93(2): 114–23. [PubMed: 12805240]
- [12]. Dvorak HF. Vascular permeability factor/vascular endothelial growth factor: a critical cytokine in tumor angiogenesis and a potential target for diagnosis and therapy. *J Clin Oncol.* 2002; 20(21): 4368–80. [PubMed: 12409337]
- [13]. Zeng H, Dvorak HF, Mukhopadhyay D. Vascular permeability factor (VPF)/vascular endothelial growth factor (VEGF) receptor-1 down-modulates VPF/VEGF receptor-2-mediated endothelial cell proliferation, but not migration, through phosphatidylinositol 3-kinase-dependent pathways. *J Biol Chem.* 2001; 276(29): 26969–79. [PubMed: 11350975]
- [14]. Millauer B, Wizigmann-Voos S, Schnurch H, Martinez R, Moller NP, Risau W, et al. High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis. *Cell.* 1993; 72(6): 835–46. [PubMed: 7681362]
- [15]. Lee CG, Heijn M, di Tomaso E, Griffon-Etienne G, Ancukiewicz M, Koike C, et al. Anti-Vascular endothelial growth factor treatment augments tumor radiation response under normoxic or hypoxic conditions. *Cancer Res.* 2000; 60(19): 5565–70. [PubMed: 11034104]
- [16]. Pham CD, Roberts TP, van Bruggen N, Melnyk O, Mann J, Ferrara N, et al. Magnetic resonance imaging detects suppression of tumor vascular permeability after administration of antibody to vascular endothelial growth factor. *Cancer Invest.* 1998; 16(4): 225–30. [PubMed: 9589031]
- [17]. Kim KJ, Li B, Winer J, Armanini M, Gillett N, Phillips HS, et al. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth *in vivo*. *Nature.* 1993; 362(6423): 841–4. [PubMed: 7683111]
- [18]. Gossman A, Helbich TH, Kuriyama N, Ostrowitzki S, Roberts TP, Shames DM, et al. Dynamic contrast-enhanced magnetic resonance imaging as a surrogate marker of tumor response to antiangiogenic therapy in a xenograft model of glioblastoma multiforme. *J Magn Reson Imaging.* 2002; 15(3): 233–40. [PubMed: 11891967]
- [19]. Kanai T, Konno H, Tanaka T, Baba M, Matsumoto K, Nakamura S, et al. Anti-tumor and anti-metastatic effects of human-vascular-endothelial-growth-factor-neutralizing antibody on human colon and gastric carcinoma xenotransplanted orthotopically into nude mice. *Int J Cancer.* 1998; 77(6): 933–6. [PubMed: 9714067]
- [20]. Konno H, Arai T, Tanaka T, Baba M, Matsumoto K, Kanai T, et al. Antitumor effect of a neutralizing antibody to vascular endothelial growth factor on liver metastasis of endocrine neoplasm. *Jpn J Cancer Res.* 1998; 89(9): 933–9. [PubMed: 9818029]
- [21]. Harmey JH, Bouchier-Hayes D. Vascular endothelial growth factor (VEGF), a survival factor for tumour cells: implications for antiangiogenic therapy. *Bioessays.* 2002; 24(3): 280–3. [PubMed: 11891765]
- [22]. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, et al. Paclitaxel-carboplatin alone or with bevacizumab for nonsmall-cell lung cancer. *N Engl J Med.* 2006; 355(24): 2542–50. [PubMed: 17167137]

- [23]. Reck M, von Pawel J, Zatloukal P, Ramlau R, Gorbounova V, Hirsh V, 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(8): 1227–34. [PubMed: 19188680]
- [24]. Sprattlin JL, Cohen RB, Eadens M, Gore L, Camidge DR, Diab S, et al. Phase I pharmacologic and biologic study of ramucirumab (IMC-1121B), a fully human immunoglobulin G1 monoclonal antibody targeting the vascular endothelial growth factor receptor-2. *J Clin Oncol*. 2010; 28(5): 780–7. [PubMed: 20048182]
- [25]. Miao HQ, Hu K, Jimenez X, Navarro E, Zhang H, Lu D, et al. Potent neutralization of VEGF biological activities with a fully human antibody Fab fragment directed against VEGF receptor 2. *Biochem Biophys Res Commun*. 2006; 345(1): 438–45. [PubMed: 16682007]
- [26]. Perol M, Ciuleanu TE, Arrieta O, Prabhaskar K, Syrigos KN, Goksel T, et al. Quality of life results from the phase 3 REVEL randomized clinical trial of ramucirumab-plus-docetaxel versus placebo-plus-docetaxel in advanced/metastatic non-small cell lung cancer patients with progression after platinum-based chemotherapy. *Lung Cancer*. 2016; 93: 95–103. [PubMed: 26898621]
- [27]. Roth GJ, Heckel A, Colbatzky F, Handschuh S, Kley J, Lehmann-Lintz T, et al. Design, synthesis, and evaluation of indolinones as triple angiokinase inhibitors and the discovery of a highly specific 6-methoxycarbonyl-substituted indolinone (BIBF 1120). *J Med Chem*. 2009; 52(14): 4466–80. [PubMed: 19522465]
- [28]. Hilberg F, Roth GJ, Krssak M, Kautschitsch S, Sommergruber W, Tontsch-Grunt U, et al. BIBF 1120: triple angiokinase inhibitor with sustained receptor blockade and good antitumor efficacy. *Cancer Res*. 2008; 68(12): 4774–82. [PubMed: 18559524]
- [29]. Kutluk Cenik B, Ostapoff KT, Gerber DE, Brekken RA. BIBF 1120 (nintedanib), a triple angiokinase inhibitor, induces hypoxia but not EMT and blocks progression of preclinical models of lung and pancreatic cancer. *Mol Cancer Ther*. 2013; 12(6): 992–1001. [PubMed: 23729403]
- [30]. Reck M, Kaiser R, Mellemaard A, Douillard JY, Orlov S, Krzakowski M, et al. Docetaxel plus nintedanib versus docetaxel plus placebo in patients with previously treated non-small-cell lung cancer (LUME-Lung 1): a phase 3, double-blind, randomised controlled trial. *Lancet Oncol*. 2014; 15(2): 143–55. [PubMed: 24411639]
- [31]. Eisenreich A Regulation of vascular function on posttranscriptional level. *Thrombosis*. 2013; 2013: 948765. [PubMed: 24288605]
- [32]. Spicer EK, Horton R, Bloem L, Bach R, Williams KR, Guha A, et al. Isolation of cDNA clones coding for human tissue factor: primary structure of the protein and cDNA. *Proc Natl Acad Sci U S A*. 1987; 84(15): 5148–52. [PubMed: 3037536]
- [33]. Goldin-Lang P, Tran QV, Fichtner I, Eisenreich A, Antoniak S, Schulze K, et al. Tissue factor expression pattern in human non-small cell lung cancer tissues indicate increased blood thrombogenicity and tumor metastasis. *Oncol Rep*. 2008; 20(1): 123–8. [PubMed: 18575726]
- [34]. Cocco E, Varughese J, Buza N, Bellone S, Glasgow M, Bellone M, et al. Expression of tissue factor in adenocarcinoma and squamous cell carcinoma of the uterine cervix: implications for immunotherapy with hI-con1, a factor VII-IgGFc chimeric protein targeting tissue factor. *BMC Cancer*. 2011; 11: 263. [PubMed: 21693061]
- [35]. Ma Z, Zhang T, Wang R, Cheng Z, Xu H, Li W, et al. Tissue factor-factor VIIa complex induces epithelial ovarian cancer cell invasion and metastasis through a monocytes-dependent mechanism. *Int J Gynecol Cancer*. 2011; 21(4): 616–24. [PubMed: 21543928]
- [36]. Cole M, Bromberg M. Tissue factor as a novel target for treatment of breast cancer. *Oncologist*. 2013; 18(1): 14–8. [PubMed: 23287882]
- [37]. Perry JR. Thromboembolic disease in patients with high-grade glioma. *Neuro Oncol*. 2012; 14 Suppl 4: iv73–80. [PubMed: 23095833]
- [38]. Thaler J, Ay C, Mackman N, Metz-Schimmerl S, Stift J, Kaider A, et al. Microparticle-associated tissue factor activity in patients with pancreatic cancer: correlation with clinicopathological features. *Eur J Clin Invest*. 2013; 43(3): 277–85. [PubMed: 23398637]

- [39]. Eisenreich A, Zakrzewicz A, Huber K, Thierbach H, Pepke W, Goldin-Lang P, et al. Regulation of pro-angiogenic tissue factor expression in hypoxia-induced human lung cancer cells. *Oncol Rep.* 2013; 30(1): 462–70. [PubMed: 23604472]
- [40]. Leppert U, Eisenreich A. The role of tissue factor isoforms in cancer biology. *Int J Cancer.* 2015; 137(3): 497–503. [PubMed: 24806794]
- [41]. Eisenreich A, Bolbrinker J, Leppert U. Tissue Factor: A Conventional or Alternative Target in Cancer Therapy. *Clin Chem.* 2016; 62(4): 563–70. [PubMed: 26896447]
- [42]. Blume-Jensen P, Hunter T. Oncogenic kinase signalling. *Nature.* 2001; 411(6835): 355–65. [PubMed: 11357143]
- [43]. Jorge SE, Kobayashi SS, Costa DB. Epidermal growth factor receptor (EGFR) mutations in lung cancer: preclinical and clinical data. *Braz J Med Biol Res.* 2014; 47(11): 929–39. [PubMed: 25296354]
- [44]. Huang SM, Harari PM. Epidermal growth factor receptor inhibition in cancer therapy: biology, rationale and preliminary clinical results. *Invest New Drugs.* 1999; 17(3): 259–69. [PubMed: 10665478]
- [45]. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science.* 2004; 304(5676): 1497–500. [PubMed: 15118125]
- [46]. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med.* 2004; 350(21): 2129–39. [PubMed: 15118073]
- [47]. Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, et al. EGF receptor gene mutations are common in lung cancers from “never smokers” and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci U S A.* 2004; 101(36): 13306–11. [PubMed: 15329413]
- [48]. Formisano L, D’Amato V, Servetto A, Brillante S, Raimondo L, Di Mauro C, et al. Src inhibitors act through different mechanisms in Non-Small Cell Lung Cancer models depending on EGFR and RAS mutational status. *Oncotarget.* 2015; 6(28): 26090–103. [PubMed: 26325669]
- [49]. Yasuda H, Kobayashi S, Costa DB. EGFR exon 20 insertion mutations in non-small-cell lung cancer: preclinical data and clinical implications. *Lancet Oncol.* 2012; 13(1): e23–31. [PubMed: 21764376]
- [50]. Yasuda H, Park E, Yun CH, Sng NJ, Lucena-Araujo AR, Yeo WL, et al. Structural, biochemical, and clinical characterization of epidermal growth factor receptor (EGFR) exon 20 insertion mutations in lung cancer. *Sci Transl Med.* 2013; 5(216): 216ra177.
- [51]. Shigematsu H, Lin L, Takahashi T, Nomura M, Suzuki M, Wistuba II, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst.* 2005; 97(5): 339–46. [PubMed: 15741570]
- [52]. Kumar A, Petri ET, Halmos B, Boggon TJ. Structure and clinical relevance of the epidermal growth factor receptor in human cancer. *J Clin Oncol.* 2008; 26(10): 1742–51. [PubMed: 18375904]
- [53]. Eck MJ, Yun CH. Structural and mechanistic underpinnings of the differential drug sensitivity of EGFR mutations in non-small cell lung cancer. *Biochim Biophys Acta.* 2010; 1804(3): 559–66. [PubMed: 20026433]
- [54]. Yun CH, Boggon TJ, Li Y, Woo MS, Greulich H, Meyerson M, et al. Structures of lung cancer-derived EGFR mutants and inhibitor complexes: mechanism of activation and insights into differential inhibitor sensitivity. *Cancer Cell.* 2007; 11(3): 217–27. [PubMed: 17349580]
- [55]. Red Brewer M, Yun CH, Lai D, Lemmon MA, Eck MJ, Pao W. Mechanism for activation of mutated epidermal growth factor receptors in lung cancer. *Proc Natl Acad Sci U S A.* 2013; 110(38): E3595–604. [PubMed: 24019492]
- [56]. Sharma SV, Bell DW, Settleman J, Haber DA. Epidermal growth factor receptor mutations in lung cancer. *Nat Rev Cancer.* 2007; 7(3): 169–81. [PubMed: 17318210]
- [57]. Costa DB, Halmos B, Kumar A, Schumer ST, Huberman MS, Boggon TJ, et al. BIM mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations. *PLoS Med.* 2007; 4(10): 1669–79; discussion 80. [PubMed: 17973572]

- [58]. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med*. 2005; 353(2): 123–32. [PubMed: 16014882]
- [59]. Thatcher N, Chang A, Parikh P, Rodrigues Pereira J, Ciuleanu T, von Pawel J, et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). *Lancet*. 2005; 366(9496): 1527–37. [PubMed: 16257339]
- [60]. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med*. 2009; 361(10): 947–57. [PubMed: 19692680]
- [61]. Fukuoka M, Wu YL, Thongprasert S, Sunpaweravong P, Leong SS, Sriuranpong V, et al. Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). *J Clin Oncol*. 2011; 29(21): 2866–74. [PubMed: 21670455]
- [62]. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol*. 2012; 13(3): 239–46. [PubMed: 22285168]
- [63]. Sequist LV, Yang JC, Yamamoto N, O’Byrne K, Hirsh V, Mok T, et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. *J Clin Oncol*. 2013; 31(27): 3327–34. [PubMed: 23816960]
- [64]. Han JY, Park K, Kim SW, Lee DH, Kim HY, Kim HT, et al. First-SIGNAL: first-line single-agent iressa versus gemcitabine and cisplatin trial in never-smokers with adenocarcinoma of the lung. *J Clin Oncol*. 2012; 30(10): 1122–8. [PubMed: 22370314]
- [65]. Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. *Lancet Oncol*. 2010; 11(2): 121–8. [PubMed: 20022809]
- [66]. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med*. 2010; 362(25): 2380–8. [PubMed: 20573926]
- [67]. Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. *Lancet Oncol*. 2011; 12(8): 735–42. [PubMed: 21783417]
- [68]. Aad G, Abbott B, Abdallah J, Abdel Khalek S, Abdinov O, Aben R, et al. Measurements of the Nuclear Modification Factor for Jets in Pb+Pb Collisions at radical(s)NN=2.76 TeV with the ATLAS detector. *Phys Rev Lett*. 2015; 114(7): 072302. [PubMed: 25763955]
- [69]. Wu YL, Zhou C, Hu CP, Feng J, Lu S, Huang Y, et al. Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non-small-cell lung cancer harbouring EGFR mutations (LUX-Lung 6): an open-label, randomised phase 3 trial. *Lancet Oncol*. 2014; 15(2): 213–22. [PubMed: 24439929]
- [70]. Yun CH, Mengwasser KE, Toms AV, Woo MS, Greulich H, Wong KK, et al. The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. *Proc Natl Acad Sci U S A*. 2008; 105(6): 2070–5. [PubMed: 18227510]
- [71]. Cross DA, Ashton SE, Ghiorghiu S, Eberlein C, Nebhan CA, Spitzler PJ, et al. AZD9291, an irreversible EGFR TKI, overcomes T790M-mediated resistance to EGFR inhibitors in lung cancer. *Cancer Discov*. 2014; 4(9): 1046–61. [PubMed: 24893891]
- [72]. Walter AO, Sjin RT, Haringsma HJ, Ohashi K, Sun J, Lee K, et al. Discovery of a mutant-selective covalent inhibitor of EGFR that overcomes T790M-mediated resistance in NSCLC. *Cancer Discov*. 2013; 3(12): 1404–15. [PubMed: 24065731]
- [73]. Zhou W, Ercan D, Chen L, Yun CH, Li D, Capelletti M, et al. Novel mutant-selective EGFR kinase inhibitors against EGFR T790M. *Nature*. 2009; 462(7276): 1070–4. [PubMed: 20033049]

- [74]. Lee HJ, Schaefer G, Heffron TP, Shao L, Ye X, Sideris S, et al. Noncovalent wild-type-sparing inhibitors of EGFR T790M. *Cancer Discov.* 2013; 3(2): 168–81. [PubMed: 23229345]
- [75]. Mok TS, Wu YL, Ahn MJ, Garassino MC, Kim HR, Ramalingam SS, et al. Osimertinib or Platinum-Pemetrexed in EGFR T790M-Positive Lung Cancer. *N Engl J Med.* 2016.
- [76]. Doebele RC, Camidge DR. Targeting ALK, ROS1, and BRAF kinases. *J Thorac Oncol.* 2012; 7(16 Suppl 5): S375–6. [PubMed: 23160323]
- [77]. Paik JH, Choi CM, Kim H, Jang SJ, Choe G, Kim DK, et al. Clinicopathologic implication of ALK rearrangement in surgically resected lung cancer: a proposal of diagnostic algorithm for ALK-rearranged adenocarcinoma. *Lung Cancer.* 2012; 76(3): 403–9. [PubMed: 22129856]
- [78]. Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature.* 2007; 448(7153): 561–6. [PubMed: 17625570]
- [79]. Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med.* 2010; 363(18): 1693–703. [PubMed: 20979469]
- [80]. Wong DW, Leung EL, So KK, Tam IY, Sihoe AD, Cheng LC, et al. The EML4-ALK fusion gene is involved in various histologic types of lung cancers from nonsmokers with wild-type EGFR and KRAS. *Cancer.* 2009; 115(8): 1723–33. [PubMed: 19170230]
- [81]. Soda M, Takada S, Takeuchi K, Choi YL, Enomoto M, Ueno T, et al. A mouse model for EML4-ALK-positive lung cancer. *Proc Natl Acad Sci U S A.* 2008; 105(50): 19893–7. [PubMed: 19064915]
- [82]. Shaw AT, Kim DW, Nakagawa K, Seto T, Crino L, Ahn MJ, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. *N Engl J Med.* 2013; 368(25): 2385–94. [PubMed: 23724913]
- [83]. Shaw AT, Kim DW, Mehra R, Tan DS, Felip E, Chow LQ, et al. Ceritinib in ALK-rearranged non-small-cell lung cancer. *N Engl J Med.* 2014; 370(13): 1189–97. [PubMed: 24670165]
- [84]. Shaw AT, Gandhi L, Gadgeel S, Riely GJ, Cetnar J, West H, et al. Alectinib in ALK-positive, crizotinib-resistant, non-small-cell lung cancer: a single-group, multicentre, phase 2 trial. *Lancet Oncol.* 2016; 17(2): 234–42. [PubMed: 26708155]
- [85]. Gainor JF, Shaw AT. Novel targets in non-small cell lung cancer: ROS1 and RET fusions. *Oncologist.* 2013; 18(7): 865–75. [PubMed: 23814043]
- [86]. Shaw AT, Ou SH, Bang YJ, Camidge DR, Solomon BJ, Salgia R, et al. Crizotinib in ROS1-rearranged non-small-cell lung cancer. *N Engl J Med.* 2014; 371(21): 1963–71. [PubMed: 25264305]
- [87]. Rafei H, El-Bahesh E, Finianos A, Nassereddine S, Tabbara I. Immune-based Therapies for Non-small Cell Lung Cancer. *Anticancer Res.* 2017; 37(2): 377–87. [PubMed: 28179283]
- [88]. Keir ME, Francisco LM, Sharpe AH. PD-1 and its ligands in T-cell immunity. *Curr Opin Immunol.* 2007; 19(3): 309–14. [PubMed: 17433872]
- [89]. Brahmer J, Reckamp KL, Baas P, Crino L, Eberhardt WE, Poddub-skaya E, et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. *N Engl J Med.* 2015; 373(2): 123–35. [PubMed: 26028407]
- [90]. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. *N Engl J Med.* 2015; 373(17): 1627–39. [PubMed: 26412456]
- [91]. Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Csoszi T, Fulop A, et al. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. *N Engl J Med.* 2016; 375(19): 1823–33. [PubMed: 27718847]
- [92]. Fehrenbacher L, Spira A, Ballinger M, Kowanzetz M, Vansteenkiste J, Mazieres J, et al. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. *Lancet.* 2016; 387(10030): 1837–46. [PubMed: 26970723]
- [93]. Rittmeyer A, Barlesi F, Waterkamp D, Park K, Ciardiello F, von Pawel J, et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3,

open-label, multicentre randomised controlled trial. *Lancet*. 2017; 389(10066): 255–65.
[PubMed: 27979383]

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