UNMET MEDICAL NEEDS IN NON-SMALL-CELL LUNG CANCER TREATMENT: HOW TO DESIGN PRE-EMPTIVE COMBINATION THERAPIES

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ABSTRACT

The rapidly expanding catalogue of human oncogenic mutations, coupled with difficulties in identifying the cellular targets of active compounds in phenotypic screens, has refocused drug discovery efforts on inhibitors of specific cellular proteins. This new 'target-based' approach has enjoyed some spectacular successes in several types of tumours, including non-small-cell lung cancer (NSCLC). Epidermal growth factor receptor (EGFR) mutations occur in 17% of NSCLC patients, with notable response to single agent therapy. Unfortunately, all patients eventually develop acquired resistance to EGFR tyrosine kinase inhibitors (TKIs), while complete remission rate to EGFR TKIs monotherapy is low. Priming BIM, a proapoptotic signalling BH3-only protein, induces sensitivity to erlotinib [Tarceva®] in EGFR-mutant cell lines. Synthetic lethal approaches and pre-emptive therapies based on the initial expression of BIM may significantly improve treatment outcomes. EGFR mutations result in transient pro-death imbalance of survival and apoptotic signalling in response to EGFR inhibition. Src homology 2 domain-containing phosphatase 2 is essential to the balance between extracellular signal-regulated kinase, phosphoinositide-3-kinase/protein kinase B and signal transducer and activator of transcription 3 activity. Furthermore, stromal hepatocyte growth factor confers EGFR TKI resistance and induces inter-receptor crosstalk with Ephrin Type-A receptor 2, CDCP1, AXL, and JAK1. A better understanding of the complex cancer molecular biology of EGFR mutant lung cancer is crucial for development of effective treatment and design of successful future clinical studies.

Keywords: Lung cancer, epidermal growth factor receptor (EGFR) mutations, synthetic lethal combinations.

INTRODUCTION

Attempts to treat cancer with drugs that target mutated proteins have been met with mixed success. Lung adenocarcinoma is a typical example in which systemic therapy is personalised based on predictive molecular biomarkers. Anti-cancer treatments are dominated by targeting genetic abnormalities such as oncogenes or non-oncogenic genetic defects. Historically, the standard of care for advanced non-small-cell lung cancer (NSCLC) has been platinum-based combination chemotherapy. This 'one-size-fits-all' approach to treatment has plateaued in terms of efficacy and has been largely supplanted by a 'personalised' approach, primarily due to the discovery that certain subsets of patients have a mutated or overexpressed receptor tyrosine kinase (RTK) gene responsible for initiation and maintenance of their cancer.[1,2] Erlotinib, gefitinib (Iressa®), and the second-generation, irreversible epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) afatinib (Giotrif®), have

offered a therapeutic alternative for patients with metastatic EGFR positive lung cancer; an approach that has proven superiority over standard platinum-based chemotherapy.[3-5]

A recent meta-analysis of patients with NSCLC and EGFR activating mutations showed that first-generation EGFR TKIs significantly delayed disease progression but had no effect on overall survival (OS).[6] However, targeting genetic defects such as EGFR mutations with a personalised strategy is limited by the high degree of intra-tumour heterogeneity, adaptation of genetic networks, and high somatic mutation rates in cancer.[7] In this short review we will try to demonstrate that, 10 years after the discovery of EGFR mutations, first-line EGFR TKI monotherapy for patients with mutant EGFR NSCLC is incomplete, and EGFR inhibitors, reversible or irreversible, are unlikely to provide cures for the majority of patients.

SYNTHETIC LETHALITY APPROACHES FOR IMPROVING SURVIVAL IN NSCLC

The Spanish Lung Cancer Group performed the first large scale screening of EGFR mutations for erlotinib treatment.[8] We were also able to examine the expression levels of the proapoptotic signalling BH3-only protein, BIM, in pretreatment tumour samples from 83 patients included in the EURTAC trial.[3] BIM expression was low or intermediate in 53 (63.96%) and high in 30 (36.14%) of these patients. Progression-free survival (PFS) to erlotinib was 12.9 months for those with high and 7.2 months for those with low/intermediate BIM expression levels, while among chemotherapy-treated patients, it was 5.8 and 5.5 months, respectively (p=0.0003).[9] OS was 28.6 months for patients with high BIM expression and 22.1 months for those with low/intermediate BIM expression (p=0.0364).[9]

Multivariate analyses showed that erlotinib was a marker of longer PFS (HR=0.35, p=0.0003), while high BIM expression was a marker of longer PFS (HR=0.49, p=0.0122) and OS (HR=0.53, p=0.0323).[9] The levels of all three major splicing isoforms - BIM extra-long (BIM-EL), BIM long, and BIM short - are induced after erlotinib treatment in drug-sensitive PC-9 cells, but not in drug-resistant H1650 and H1975 cells. EGFR signalling influences BIM expression and phosphorylation status mainly via the ERK pathway, and erlotinib appears to induce significant dephosphorylation of BIM-EL, resulting in an increase in its pro-apoptotic function.[10,11] Although pretreatment BIM expression levels may not be enough to predict outcome to EGFR TKIs, they may serve as a companion diagnostic marker for synthetic lethal combinations in lung cancer with EGFR mutations.

RESISTANCE TO EGFR TKIS, EVEN WITH HIGH BASELINE BIM LEVELS

The two primary signalling pathways activated by EGFR include the mitogen-activated protein kinase (MAPK) and phosphoinositide-3-kinase (PI3K) axes. Src tyrosine kinases and activation of the signal transducer and an activator of the transcription 3 (STAT3) pathway, as well as downstream signalling, have also been well documented.[12] EGFR phosphorylation leads to recruitment of multiple effecter proteins through recognition and binding of Src-homology 2 domain-containing phosphatase 2 (SHP2) to phosphotyrosine motifs on the receptor.[12] SHP2 (encoded by PTPN11) is a ubiquitously expressed SH2 domain-containing protein tyrosine phosphatase. Despite its direct function in protein dephosphorylation, SHP2 plays an overall positive role in transducing signals initiated from growth factors/cytokines and extracellular matrix proteins and initiating various downstream signalling cascades, including the PI3K and MAPK.[12,13] By contrast, SHP2 functions as a negative regulator of the Janus kinase (JAK)/STAT pathway.[14]

In 2004, Sordella and colleagues[15] were able to demonstrate the differential EGF-induced tyrosine phosphorylation pattern seen with wild-type (WT) and mutant EGFR receptors. For instance, Y845 is highly phosphorylated in the L858R missense mutant, but not in the WT or deletion mutant, and hence, appears to be unique in distinguishing between the two types of EGFR mutations.[15] Y845 (pY845) phosphorylation stabilises the activation loop, maintains the enzyme in an active state, and regulates STAT3/5 activity.[15] Surprisingly, the EGFR L858R mutation leads to a decreased ability to activate ERK compared to WT EGFR, which correlates with decreased EGFR internalisation, reduced phosphorylation of SHP2, hyperactivity of STAT3, and reduced sensitivity to gefitinib.[16] Lazzara and colleagues[16] found that SHP2 Y542 phosphorylation was not induced in response to EGF in the H3255 cells, which harbour the missense L858R exon 21 mutation, suggesting that SHP2 activity may be less efficiently promoted by EGFR L858R and the STAT3 pathway may be more active.

The main problem is that STAT3 signalling is not inhibited with EGFR TKI monotherapy.[15] According to our experience, the combination of gefitinib + ruxolitinib (Jakavi®: a JAK inhibitor) is additive in the 11-18 cell line which also harbours the EGFR L858R mutation (unpublished data). Even the second-generation irreversible EGFR TKIs, such as afatinib or dacomitinib, do not abrogate, and may also induce STAT3 phosphorylation in gefitinib or erlotinib-resistant cell lines such as H1975 or PC9-R.[17] Afatinib activates interleukin-6 receptor (IL-6R)/JAK1/STAT3 signalling via autocrine IL-6 secretion in both cells. Blockade of IL-6R/JAK1 significantly increases sensitivity to afatinib through inhibition of afatinib-induced STAT3 activation. The role of the paracrine IL-6R/JAK1/STAT3 loop between stroma and cancer cells in the development of drug resistance is crucial.[17] Yao et al.[18] uncovered the existence of a subpopulation of cells, intrinsically resistant to erlotinib, which display features suggestive of epithelial-to-mesenchymal transition in NSCLC-derived cell lines and early-stage tumours before erlotinib treatment. Activation of TGF-β-mediated signalling was sufficient to induce these phenotypes. Increased TGF-β-dependent IL-6 secretion released previously addicted lung tumour cells from their EGFR dependency. Therefore, both tumour cell-autonomous mechanisms and/or activation of the tumour microenvironment could contribute to primary and acquired erlotinib resistance and, as such, treatments based on EGFR inhibition may not be sufficient for effective treatment of EGFR mutated lung cancer patients.[18] Furthermore, tumour cells exposed to reversible or irreversible EGFR TKIs display early resistance dependent on MET-independent activation of B cell lymphoma-2 (BCL-2)/BCL-extra-large (BCL-XL) survival signalling.[19]

According to the study of Fan and colleagues,[19] such cells display a quiescence-like state that is readily reversed after withdrawal of targeted inhibitors. BCL-2 induction and p-STAT3 (Y705) activation are found within the residual tumour cells surviving the initial antitumour response to targeted therapies. Niclosamide (Niclocide®) is a teniacide in the anthelmintic family, approved by the US FDA for the treatment of tapeworms.[20] This safe, inexpensive drug, used in humans for nearly 50 years, reduces expression of the transcription factor STAT3.[20] Microtubule-targeted drugs, such as paclitaxel (Taxol®), inhibit cytokine-induced STAT3 and disrupt the interaction of STAT3 with tubulin.[21] Docetaxel (Taxotere®) or paclitaxel, act as STAT3 inhibitors, shedding light on 'flare' after stopping EGFR TKIs and indicating that addition of taxanes can have a benefit through STAT3 inhibition.[21] Finally, the JAK inhibitors AZD1480 or ruxolitinib block STAT3 signalling, resulting in suppression of tumour cell growth and survival.[22] Combining EGFR TKIs (reversible or irreversible) with STAT3 inhibitors or upstream (pan-JAK) or downstream (BCL-2/BCL-XL) constituents can be more efficient in inducing apoptosis, regardless of MAPK/ERK abrogation, for EGFR mutant patients with high levels of BIM possibly related to early adaptive resistance (Figure 1).[17-19]

NSCLC EGFR MUTANT PATIENTS WITH LOW BIM LEVELS AT BASELINE

BIM expression in treatment naïve cancers predicts responsiveness to EGFR TKIs, but almost two-thirds of patients have low BIM mRNA levels at baseline.[9] SHP2, which is downstream of EGFR and several other tyrosine kinase receptors, is required for sustained activation of ERK and BIM downregulation.[23,24] Upon activation of MET by its ligand hepatocyte growth factor (HGF), provided by stromal cells, EGFR signalling is dramatically altered.[25] HGF anticipates the mode of action in EGFR mutant tumours since EGFR tyrosine kinase activity, along with classical downstream signalling, is no longer required for tumour growth.[25] Specifically, HGF confers EGFR TKI resistance by inducing two novel cancer-promoting functions: firstly, it abolishes classical EGFR signalling, which makes cancer cells independent of these signalling mechanisms and neutralises the point of action for EGFR TKI-targeted drugs. Secondly, it enables EGFR to interact with proteins known to be markers of a highly metastatic phenotype such as the CUB domain containing protein 1 (CDCP1), Ephrin Type-A Receptor 2 (EphA2), JAK1, AXL, and Mer, interactions that cannot be affected by EGFR TKI treatment. EphA2 is a member of the erythropoietin-producing hepatocellular (Eph) family of RTKs. Unlike traditional oncogenes that often function only in tumour cells, EphA2 mediates cell-cell interactions both in tumour cells and tumour stroma and vasculature. EphA2 is often overexpressed in a variety of malignant cancers, including breast, lung, prostate, and colon cancers.[23]

EphA2 phosphorylates Tyr542 and Tyr580 of SHP2 to enhance and prolong ERK activation downstream of RTKs in the cells stimulated with

growth factors, such as EGF, HGF, or Gas6.[23] Miura et al.[23] were able to demonstrate that prolonged and enhanced ERK activation in cells stimulated with growth factors was reduced in cells depleted of EphA2 with simultaneous reduction of Tyr542/580 phosphorylation.

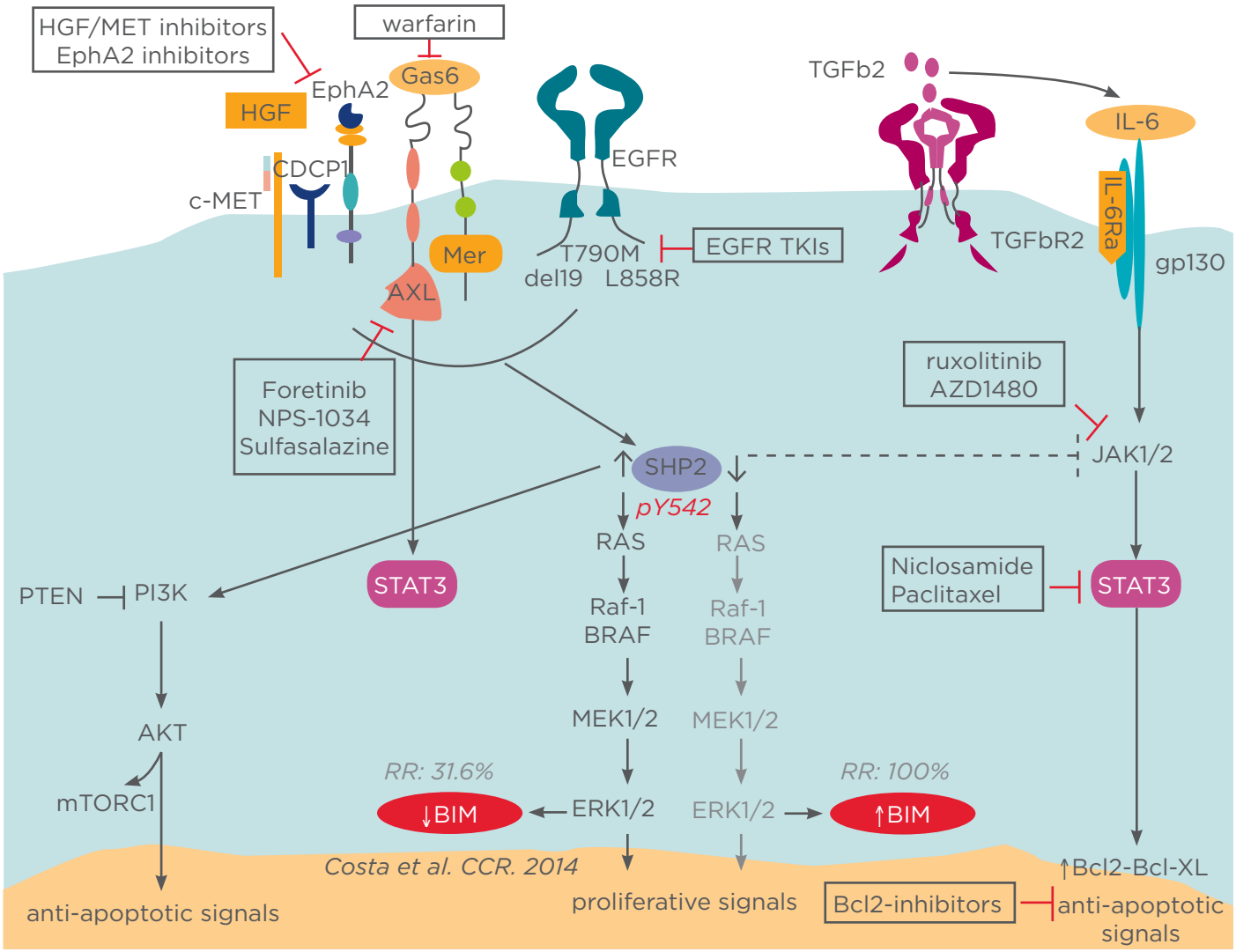

Figure 1: Potential mechanisms of resistance to EGFR tyrosine kinase inhibitors (TKIs).
The main signalling pathways activated by epidermal growth factor receptor (EGFR) include the mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), and signal transducer and activator of transcription 3 (STAT3) axes. Src homology 2 domain-containing phosphatase 2 (SHP2) plays a positive role in transduction of signals initiated from growth factors/cytokines, and extracellular matrix proteins, and initiation of various downstream signalling cascades. By contrast, SHP2 functions as a negative regulator of the Janus kinase (JAK)/STAT pathway. Combining EGFR TKIs with STAT3 inhibitors or upstream or downstream (BCL-2/BCL-XL) constituents can be more efficient in inducing apoptosis, regardless of MAPK/extracellular signal-regulated kinase (ERK) abrogation, in EGFR mutant patients with high levels of BIM, possibly related to early adaptive resistance. Combining EGFR TKIs with MET, AXL, or ephrin type-A receptor 2 (EphA2) inhibitors can be a rational and innovative synthetic lethality approach for EGFR mutant non-small-cell lung carcinoma (NSCLC) patients with low baseline BIM expression and high SHP2 activity.

HGF: hepatocyte growth factor; Gas6: growth arrest-specific 6; TGFb2: transforming growth factor-beta 2; IL: interleukin; CDCP1: CUB domain-containing protein 1; PTEN: phosphatase and tensin homologue; AKT: serine/threonine protein kinase; mTORCI: mammalian target of rapamycin complex 1; Raf: rapidly accelerated fibrosarcoma; BCL-2: B cell lymphoma 2; XL: extra-large; BIM: B-cell lymphoma 2 interacting mediator of cell death; MEK: MAPK/ERK kinase.

SHP2-dependent ERK activation signal pathway was hyperactivated, promoting cancer cell proliferation in tumours with EphA2 overexpression, measured by mRNA or immunohistochemistry.[23] Thus, treatment with HGF/MET inhibitors, together with EGFR-targeted therapies, and targeting HGF/MET-induced EGFR interactors may be necessary for the elimination of tumour growth.[25] At the same time, Gas6/AXL-mediated stimulation of ERK is attributed, in part, to its ability to activate SHP2.[24] There are several AXL or Gas6 inhibitors that can be combined with EGFR TKIs as preventive synthetic lethal therapies. Interestingly, warfarin prevents γ-carboxylation of TAM ligands, rendering Gas6 unable to activate TAM receptors (AXL and Mer).[26] Foretinib (GSK1363089), NPS-1034 but also sulfasalazine (Salazopyrin®), a synthetic nonsteroidal anti-inflammatory drug commonly used in the management of inflammatory bowel diseases and rheumatoid arthritis, are potent AXL inhibitors.[27-30]

Combining EGFR TKIs with MET, AXL, or EphA2 inhibitors can be a rational and innovative synthetic lethality approach for EGFR mutant NSCLC patients with low baseline BIM expression and high SHP2 activity. It seems that immunohistochemical staining and mRNA expression of SHP2 are well correlated and can be used as a biomarker for response.[31,32] Interestingly, STAT3 signalling can be hyperactive due to upstream pathways including not only IL-6 and JAK but also AXL, providing further opportunities for combination therapies (Figure 1).

CONCLUSIONS

Targeting genetic defects using a personalised strategy is limited by the high degree of intra-tumour heterogeneity, adaptation of genetic networks, and high somatic mutation rates in cancer. If we wish to radically change treatment of EGFR mutant NSCLC to the benefit of our patients, we should start thinking about a different approach based on information derived from additional biomarkers. BIM may serve as a companion diagnostic marker for successful synthetic lethal combinations. Patients with high BIM levels at baseline may have a hyperactive JAK/STAT pathway through either the L858R mutation or loss of SHP2 activity. The combination of EGFR TKIs + a JAK, STAT3, or BCL-2/BCL-XL inhibitor should be seriously considered in these cases. Patients with low BIM levels at baseline may benefit from the combination of EGFR TKIs with compounds that downregulate or abrogate activity of SHP2, such as MET, AXL, or EphA2 inhibitors. It should be seriously considered whether, at time of progression, a JAK or a STAT3 inhibitor could be added in order to overcome loss of the negative impact of SHP2 on the JAK/STAT3 pathway. We propose this line of research at the level of cell lines and xenograft models, and at the level of biomarker discovery in tumour samples, in order to verify our assumptions as accurately as possible and contribute to radically transforming treatment of EGFR mutant lung cancer.

REFERENCES

1. Bonanno L et al. Role of genotyping in non-small cell lung cancer treatment: current status. Drugs. 2011;71(17):2231-46.

2. Thomas A et al. Comparative effectiveness, personalized medicine and innovation: the path forward. PharmacoEconomics. 2010;28(10): 923-30.

3. Rosell R 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.

4. Mok TS et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med. 2009;361(10):947-57.

5. Sequist LV 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.

6. Lee CK et al. Impact of EGFR inhibitor in non-small cell lung cancer on progression-free and overall survival: a meta-analysis. J Natl Cancer Inst. 2013;105(9):595-605.

7. Rosell R et al. Genetics and biomarkers in personalisation of lung cancer treatment. Lancet. 2013;382(9893):720-31.

8. Rosell R et al. Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med. 2009;361(10): 958-67.

9. Costa C et al. The impact of EGFR T790M mutations and BIM mRNA expression on outcome in patients with EGFR-mutant NSCLC treated with erlotinib or chemotherapy in the randomized phase III EURTAC trial. Clin Cancer Res. 2014;20(7):2001-10.

10. Gong Y et al. Induction of BIM is essential for apoptosis triggered by EGFR kinase inhibitors in mutant EGFR-dependent lung adenocarcinomas. PLoS Med. 2007;4(10):e294.

11. Costa DB et al. BIM mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations. PLoS Med. 2007;4(10):1669-79.

12. Dasari VR et al. Cord blood stem cells inhibit epidermal growth factor receptor translocation to mitochondria in glioblastoma. PloS One. 2012;7(2):e31884.

13. Yu B et al. Targeting protein tyrosine phosphatase SHP2 for the treatment of PTPN11-associated malignancies. Mol Cancer Ther. 2013;12(9):1738-48.

14. You M et al. Shp-2 tyrosine phosphatase functions as a negative regulator of the interferon-stimulated Jak/STAT pathway. Mol Cell Biol. 1999;19(3):2416-24.

15. Sordella R et al. Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. Science. 2004;305(5687):1163-7.

16. Lazzara MJ et al. Impaired SHP2-mediated extracellular signal-regulated kinase activation contributes to gefitinib sensitivity of lung cancer cells with epidermal growth factor receptor-activating mutations. Cancer Res. 2010;70(9):3843-50.

17. Kim SM et al. Activation of IL-6R/JAK1/STAT3 signaling induces de novo resistance to irreversible EGFR inhibitors in non-small cell lung cancer with T790M resistance mutation. Mol Cancer Ther. 2012;11(10):2254-64.

18. Yao Z et al. TGF-beta IL-6 axis mediates selective and adaptive mechanisms of resistance to molecular targeted therapy in lung cancer. Proc Natl Acad Sci U.S.A. 2010;107(35):15535-40.

19. Fan W et al. MET-independent lung cancer cells evading EGFR kinase inhibitors are therapeutically susceptible to BH3 mimetic agents. Cancer Res. 2011;71(13):4494-505.

20. Londoño-Joshi AI et al. Effect of niclosamide on basal-like breast cancers. Mol Cancer Ther. 2014;13(4):800-11.

21. Walker SR et al. Microtubule-targeted chemotherapeutic agents inhibit signal transducer and activator of transcription 3 (STAT3) signaling. Mol Pharmacol. 2010;78(5):903-8.

22. Scuto A et al. The novel JAK inhibitor AZD1480 blocks STAT3 and FGFR3 signaling, resulting in suppression of human myeloma cell growth and survival. Leukemia. 2011;25(3):538-50.

23. Miura K et al. Involvement of EphA2-mediated tyrosine phosphorylation of Shp2 in Shp2-regulated activation of extracellular signal-regulated kinase. Oncogene. 2013;32(45):5292-301.

24. Maroun CR et al. The tyrosine phosphatase SHP-2 is required for sustained activation of extracellular signal-regulated kinase and epithelial morphogenesis downstream from the met receptor tyrosine kinase. Mol Cell Biol. 2000;20(22):8513-25.

25. Gusenbauer S et al. HGF induces novel EGFR functions involved in resistance formation to tyrosine kinase inhibitors. Oncogene. 2013;32(33):3846-56.

26. Paolino M et al. The E3 ligase Cbl-b and TAM receptors regulate cancer metastasis via natural killer cells. Nature. 2014;507(7493):508-12.

27. Eder JP et al. A phase I study of foretinib, a multi-targeted inhibitor of c-Met and vascular endothelial growth factor receptor 2. Clin Cancer Res. 2010;16(13):3507-16.

28. Ye X et al. An anti-Axl monoclonal antibody attenuates xenograft tumor growth and enhances the effect of multiple anticancer therapies. Oncogene. 2010;29(38):5254-64.

29. Lay JD et al. Sulfasalazine suppresses drug resistance and invasiveness of lung adenocarcinoma cells expressing AXL. Cancer Res. 2007;67(8):3878-87.

30. Rho JK et al. MET and AXL inhibitor NPS-1034 exerts efficacy against lung cancer cells resistant to EGFR kinase inhibitors because of MET or AXL activation. Cancer Res. 2014;74(1):253-62.

31. Xu R et al. Overexpression of Shp2 tyrosine phosphatase is implicated in leukemogenesis in adult human leukemia. Blood. 2005;106(9):3142-9.

32. Dong S et al. Expression and clinical significance of SHP2 in gastric cancer. J Int Med Res. 2012;40(6):2083-9.