

A critical question for cancer therapy: what new targets exist?

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Abstract: Designing molecular targeted therapy with high specificity based on novel tumor biomarkers is a high priority in lung cancer research. Several molecular aberrations have been already identified in non-small cell lung cancer (NSCLC), with subsequent development of drugs targeted to these aberrations. A more recent actionable target is MET, a multifaceted receptor tyrosine kinase which frequently interacts with other key oncogenic tyrosine kinases including epidermal growth factor receptor (EGFR) and ERBB3 leading to resistance to anti-EGFR therapies. However a phase III trial enrolling only patients with MET-positive tumors was stopped in early March due to futility since there was no evidence that the addition of onartuzumab to erlotinib has any positive effect. From the results of the MET lung phase III trial, we provide new pieces of information that can contribute to further preclinical validation and also be part of the armamentarium for clinical translational research.

Keywords: Epidermal growth factor receptor (EGFR); non-small cell lung cancer (NSCLC); MET; targeted therapy

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Lung cancer is still a common disease with dismal prognosis. However, several driver lesions have been identified which can permit the use of targeted therapy. Epidermal growth factor receptor (EGFR) mutations, ALK and ROS1 translocations have become part of the molecular diagnosis of patients, particularly in lung adenocarcinoma (1). Triple negative lung adenocarcinomas (EGFR, ALK and ROS1 negative) should be examined for other potentially druggable mutations such as HER2, BRAF, PIK3CA and NRAS among others (2). KRAS is also a common alteration for which no specific therapy yet exists. We still do not know how to take advantage of the fact that many non-small cell lung cancers (NSCLC) exhibit oncogenic kinase signaling through several receptor protein tyrosine kinases (RTKs), not only EGFR but also MET and RON, EPHA2, AXL, RET, TRKA and FGFR1 (3). In NSCLC, MET can be overexpressed along with hepatocyte growth factor (HGF).

Several MET inhibitors (4) have been tested in combination with EGFR inhibitors. However, no difference in overall survival (OS) was observed either with the combination of tivantinib plus erlotinib *vs.* erlotinib alone (5). Neither was any benefit shown in OS with an anti-MET antibody (onartuzumab) in combination with erlotinib in a phase II randomized trial (6). The same authors did not find any difference in OS in the phase III randomized trial in MET-positive NSCLC (7). However, it is possible that a subset of MET expressing tumors can respond to anti-MET therapeutics as has been recently demonstrated in some NSCLC cell lines (8). Crosstalk of MET with its family member RON (3) has been observed (*Table 1, Figure 1*). MET/RON complexes are present on the cell surface and ligand-stimulated MET activation results in direct transphosphorylation of RON (21). A MET/RON dual kinase inhibitor (LY2801653) was more efficacious than

Table 1 Potential novel targets in lung cancer

Signaling	Biomarker	Subtype of lung cancer	Available or potential targeted therapies	Evidence	
				Preclinical	Clinical
MAPK/PI3K/STAT3	MET	EGFR mutant ADC resistant to EGFR TKIs	Tivantinib + erlotinib (negative trial), onartuzumab + erlotinib (negative trial)	(8)	(5-7)
MAPK/PI3K/STAT3/ CBL	MET/RON	KRAS (G12S) mutations PDGFR amplification MET amplification	LY2801653	(8)	–
Maintenance of PI3K/AKT activity	EGFR/MET/ERBB3	EGFR mutant ADC resistant to EGFR TKIs	MET inhibitors	(9)	–
	IGFR1/EGFR	EGFR mutant ADC resistant to EGFR TKIs	Figitumumab + chemotherapy (negative trial), OSI-906	(10)	(11)
	loss of TROP2	SCC	+/- erlotinib (maintenance, NCT01186861)		
	TrkB (NTRK2)	wt EGFR ADC wt KRAS ADC	K252a (pan Trk inhibitor), ANA-12 (TrkB specific inhibitor)	(12)	(12) (predictive of metastases)
STAT3	RET/ASCL1 (TUBB2B)	ADC with NE differentiation	Sunitinib, vandetanib, cabazitaxel	–	(13) (ASCL1, marker for NE differentiation)
MAPK/PI3K/STAT3	EPHA2	ADC with KRAS mutations SCC	Dasatinib	(14)	–
MAPK/PI3K/STAT3	DDR2/SHP2	SCC	Imatinib, nilotinib, dasatinib	(15)	–
PKC δ /ERK	FGF2-FGFR1- brachyury-API5-BIM	EGFR mutant ADC resistant to EGFR SCC	FGFR tyrosine kinase inhibitors, monoclonal antibodies, and an FGF ligand trap	(16-19)	(20)

EGFR, epidermal growth factor receptor.

crizotinib (a MET/ALK/RON/ROS inhibitor) in A549 (KRAS G12S), H1703 (PDGFRA amplified), and H1993 (MET amplified) NSCLC cell lines. Also LY2801653 was effective in in vivo models. Inhibition of MET and RON was associated with decreased phosphorylation of CBL, PI3K and STAT3 (8). Since NSCLC is a heterogeneous group of diseases it is important to understand which biomarkers can model the activity of MET and specific inhibition of MET and RON could become clinically relevant, even in tumors harboring KRAS mutations. It is important to highlight that co-activation of several RTKs occur in many tumors. For example, the A549 NSCLC cell line coexpresses EGFR, MET, ERBB3, EPHA2 and AXL. The 8988T pancreatic adenocarcinoma cell line coexpresses EGFR, MET, ERBB2, RON, INSR, EPHA2 and AXL (22). The type of ALK

inhibitor also matters in the mechanism of resistance that can be developed in EML4-ALK NSCLC cells. Paracrine receptor activation by ligands from the microenvironment may trigger resistance to ALK inhibitors in EML4-ALK lung cancer cells. Fibroblasts produce HGF which activates MET/Gab1 and triggers resistance to TAE684 but not to crizotinib which also inhibits MET, as explained above). Conversely, endothelial cells which produce EGFR ligands decrease sensitivity to crizotinib (23).

Intriguingly, in MET amplified cells, MET signaling through ERBB3 maintains PI3K/AKT cell survival signaling despite EGFR inhibition (24). In spite of the negative studies combining MET inhibitors with erlotinib, it has been shown that EGFR-MET signaling is critical for aggressive behavior of NSCLC and provides the basis for further investigations

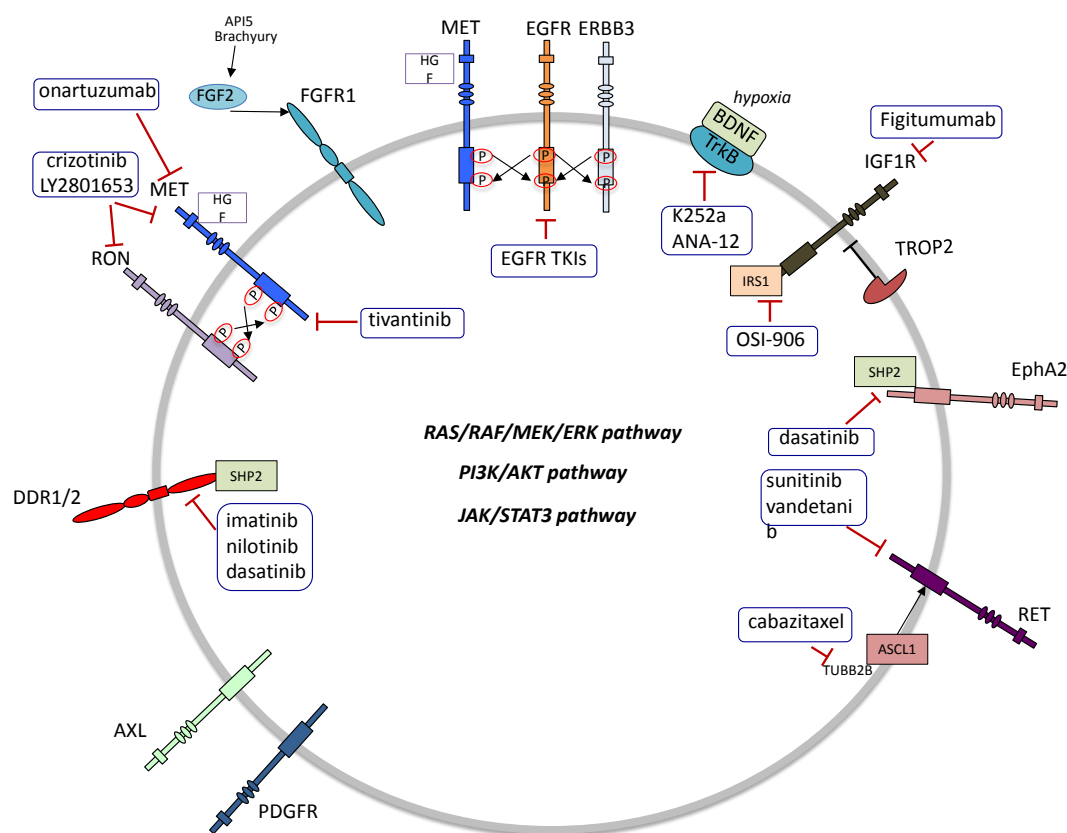


Figure 1 The crosstalk between receptor tyrosine kinases-potential novel therapeutic targets.

into therapeutic target combinations. It has been determined that EGFR activation by ligand or mutation is sufficient to induce MET phosphorylation. In addition, ERBB3 enhances EGFR-driven phosphorylation of MET and activates MET itself (9). A seminal study identified two pathways leading to PI3K/AKT signaling in A431 gefitinib resistant cells: the EGFR/ERBB3 and the IGF1R/IRS1 pathways. Combining therapeutic inhibition of EGFR and IGF1R abrogates this acquired mechanism of drug resistance (10) (Table 1, Figure 1). IGF1R inhibitors are reviewed in Gold *et al.* (11). TROP2 modulates IGF-1R signaling in lung adenocarcinoma and low levels of expression of TROP2 in NSCLC cells are related to resistance to EGFR TKIs (25). Intriguingly, IGF1R/PI3K signaling is enhanced in resistant melanomas and combined treatment with IGF1R/PI3K and MEK inhibitors induced death of BRAF inhibitor-resistant cells (26).

Tropomyosin-related kinase B (TrkB) (3) expression is regulated by hypoxia-inducible factor 1 (HIF-1) and TrkB is required for AKT activation during lung tumor cell migration. Importantly, TrkB expression is more

frequent in NSCLC wild-type for KRAS and EGFR. These observations suggest that TrkB could be an alternative way for tumors to enhance PI3K signaling. Therefore targeting TrkB could be a useful strategy in patient subsets for whom there is no currently available targeted therapy (12). Moreover, ASCL1 and RET expression define a clinically relevant subgroup of 10% of lung adenocarcinomas characterized by neuroendocrine differentiation. ASCL1 acts upstream of RET. Also, STAT3 levels are reduced in ASCL1 depleted cells, suggestion potential activation of the JAK/STAT3 pathway. Currently available drugs targeting RET, such as sunitinib or vandetanib, could be appropriate for this subgroup of patients, as well as drugs targeting TUBB2B such as cabazitaxel since TUBB2B is also associated with high levels of ASCL1 (13) (Table 1, Figure 1).

Also of great clinical relevance is the fact that EPHA2 expression (3) is increased in patients harboring KRAS mutations (27). EPHA2 expression also positively correlates with history of smoking and poor survival (27). Therefore, EPHA2 is a therapeutic target for NSCLC and dasatinib is a multi-target kinase inhibitor with significant activity

against EPHA2 (14). EPHA2 mutations have been reported in squamous cell lung carcinoma (SCC) (28). Discoidin domain receptors, particularly DDR2 (3), are activated in lung cancer and DDR2 mutations have been reported in lung SCC (29). Inhibition of DDR1 and 2 can be achieved with different multi-target kinase inhibitors such as imatinib, nilotinib and dasatinib (15). SHP2 is a key signaling node downstream of the DDR2 receptor which leads to activation of multiple signaling pathways (29) (*Table 1, Figure 1*).

FGFR expression also matters in lung cancer (3). An important new finding is that FGFR1 mRNA levels may serve as a better biomarker of FGFR1 TKI response in lung cancer than FGFR1 gene copy number (30). Also, activation of FGF2-FGFR1 was described as a mechanism of acquired resistance to gefitinib in NSCLC (16). Brachyury, described as a driver of epithelial to mesenchymal transition, was reported to be overexpressed in NSCLC and suggested to offer an opportunity for novel therapeutic interventions (17). More recently, it has been shown that FGFR phosphorylation activates MEK/ERK resulting in increased Brachyury expression. Brachyury in turn promotes secretion of FGF and enhances again FGF-FGFR signaling (18). Brachyury levels could be a new biomarker for therapeutic interventions with FGF pathway inhibitors. The list of inhibitors is reviewed in Corn *et al.* (20). Also the anti-apoptotic gene AP15 mediates resistance by upregulating FGF2 signaling through FGFR1/PKC δ /ERK effector pathway which triggers degradation of the pro-apoptotic molecule BIM (19) (*Table 1, Figure 1*).

In summary, in NSCLC patients pan-negative for druggable driver genetic alterations, selection for multi-target kinase inhibitors also warrants selection based on the expression of one or more than one RTKs (3,22). In addition, several new biomarkers could be candidates for incorporation in customizing treatment for NSCLC patients negative for the most common driver mutations.

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