Editorial

Inhibition of MEK, a canonical KRAS pathway effector in *KRAS* mutant NSCLC

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KRAS mutant NSCLC cells require active nuclear export of Iκβα (also known as NFKBIA), a negative regulatory protein of NF-κB signaling, for maintaining survival signaling (1-3). Nuclear export receptor XPO1 correlates with *KRAS* mutation status. Sensitivity to XPO1 inhibitors (KPT-330 or Selinexor) is associated with apoptosis in *KRAS* mutant cell lines. In contrast, chemical inhibition of mitogen-activated protein kinase kinase (also known as MEK) has little consequence on cell viability (1). XPO1 inhibitors induce the nuclear accumulation of Iκβα in a broad panel of tested cell lines, indicating that selective sensitivity is related to inhibition of NF-κB signaling (4). Jänne *et al.* (5) carried out the phase 3 Selumetinib Evaluation as Combination Therapy (SELECT-1) trial which assessed second line selumetinib plus docetaxel for patients with *KRAS* mutant, metastatic NSCLC versus placebo plus docetaxel. The SELECT-1 trial did not improve progression free survival (PFS) or overall survival (OS). Median PFS was 3.9 months in the selumetinib plus docetaxel group and 2.8 months in the placebo plus docetaxel group. Median OS was 8.7 months in the selumetinib plus docetaxel group versus 7.9 months in the placebo plus docetaxel group. The Jänne *et al.* study highlights many aspects of the difficulties in treating *KRAS* mutant NSCLC patients. The meager effect of selumetinib as a MEK inhibitor should be revisited based upon the abundant information reaped from the study to move forward from bench to bed. Undeniably, there are multiple approaches. Firstly, KRAS protein induced XPO1-dependent activation of NF-κB signaling in NSCLC cells (1) should be explored. This activation is not required for wild-type tumor NSCLC lines and XPO1 inhibitors warrant testing in the clinical setting. Noteworthy is the fact that *FSTL5* mutations found in *KRAS* mutant cell lines were resistant to XPO1 inhibitors. Somatic mutations in *FSTL5* are found in 10% of lung adenocarcinomas. *FSTL5* depletion produces sensitivity to XPO1 inhibitors in *KRAS* mutant, *FSTL5* wild-type NSCLC cell lines. Notably, *FSTL5* depletion induces YAP1 activation, akin to that induced upon depletion of the *LATS1* and *LATS2* tumor suppressor genes (1). There is strong evidence between the *FSTL5* mutation status and YAP1 protein accumulation. Intriguingly, we show that an increase in YAP1 in BRAF and *KRAS* mutant NSCLC tumors is a biomarker predicting worse response to RAF and MEK inhibition in patients (6). Secondly, it has been reported that the IκB kinase (IKK)-related kinases TANK-binding kinase-1 (TBK1) and IKKε promote KRAS driven activity by regulating interleukin (IL)-6 and identify CYT387 as a potent JAK/TBK1/IKKε inhibitor (7). Thirdly, MEK inhibitors are clinically active in $BRAF^{v600E\ mutant}$ melanomas, but only marginally active in *KRAS* mutant tumors. MEK inhibitors induce RAF-MEK complexes in *KRAS* mutant models and disrupting such complexes enhanced inhibition of RAF proto-oncogene serine/threonine-protein kinase (CRAF)—dependent extracellular signal-regulated kinase (ERK) signaling (8).

In fact, ablation of CRAF expression induces regression of *KRAS-Trp53* mutant lung tumors (9). The combination of sorafenib [a multi-kinase inhibitor that targets both, CRAF and BRAF, as well as vascular endothelial growth factor receptor (VEGFR)] and aspirin in *KRAS* mutant NSCLC cells produces a significant reduction of cell proliferation within 72 hours in A549 and H358 cells by simultaneously effecting two independent pathways when the tumor cells were sensitive to single agents, sorafenib and aspirin (10). Although trametinib is superior to other MEK inhibitors since it impairs feedback reactivation of ERK, it activates multiple signaling pathways, reflecting a relief in feedback mechanisms produced by hyperactive KRAS signaling in *KRAS* mutant NSCLC cells (11,12). Trametinib, as other MEK inhibitors, activates signal transducer and activator of transcription 3 (STAT3), as well as several receptor tyrosine kinases (RTKs), including fibroblast growth factor receptor 1 (FGFR1) and the FGFR adaptor protein, fibroblast growth factor receptor substrate 2 (FRS2) (11,13). The sensitivity to the combination of trametinib and FGFR inhibition (ponatinib) correlates with the degree of FRS2 phosphorylation after trametinib treatment (11). Intriguingly, in combination with trametinib, afatinib shows activity in *KRAS* mutant NSCLC lines (11) in accordance with other findings that epithelial *KRAS* mutant NSCLC cell lines overexpress ERBB3 and are sensitive to the combination of afatinib plus a MEK inhibitor, while mesenchymal *KRAS* mutant NSCLC cell lines following MEK inhibition overexpress FGFR1 and FRS2, and, henceforth, are sensitive to the combination of a MEK inhibitor plus an FGFR inhibitor (NVP-BGJ398) (14). The fact that activation of YAP1 stimulates secretion of FGF ligands and expression of FGFR in ovarian cancer is significant (15). Different lines of evidence show that, following MEK inhibition, there could be overexpression of other RTKs, like MET and AXL, as well as overactivation of Src-YAP1-NOTCH-HES1, in addition to STAT3 (16,17). AXL overexpression has been a trait of *KRAS* mutant cell lines with mesenchymal features responding to the combination of erlotinib and an AXL inhibitor (18), or the combination of the AXL inhibitor, TP0903, plus a PARP inhibitor (olaparib) (19). Inhibition of AXL directly reverts the epithelial-mesenchymal transition (EMT) phenotype and leads to decreased expression of DNA repair genes, diminishing homologous recombination proficiency (19). The combination of a WEE1 inhibitor with an MTOR inhibitor has been reported in mutant *KRAS* NSCLC tumors (20). The combination of MEK inhibitors with Src inhibitors could be of great interest, since a transmembrane protein, CUB domain-containing proteins (CDCP1), is required for the functional link between RAS and Src signaling. Most *KRAS* mutant NSCLC tumors overexpress CDCP1 (21). CDCP1 can also interact with and activate all Src-family kinase (SFK) members, such as, YES and LYN (17,22). At least 21% of c NSCLCs show significant integrin β3 (*ITGB3*) mRNA expression and targeting galectin-3 could be a novel strategy for such *KRAS* mutant tumors addicted to integrin αvβ3/galectin-3 (GCS-100) (23).

Loss of function of MutT homolog 1 (MTH1), a nucleotide pool sanitizing enzyme, impairs growth of *KRAS* mutant tumor cells. Overexpression of *MTH1* mRNA levels has been shown to be a prognostic factor, documented in lung cancer and renal cell carcinoma, and MTH1 inhibitors are in development. It was found that (S)-crizotinib efficiently inhibited colony formation of *KRAS* mutated cells, like an MTH1 inhibitor (SCH51344). (S)-crizotinib is less potent than the (R)-enantiomer against the established anaplastic lymphoma kinase (ALK), MET and ROS1 (24).

Justilien and Fields describe the relevance of protein kinase Cι (PKCι) in *KRAS* mutant NSCLC, activating a RAC1-PAK-MEK1,2-ERK1,2 signaling pathway and show that epithelial cell transforming sequence 2 (Ect2), a guanine nucleotide exchange factor for Rho family GTPases is amplified and overexpressed with PKCι in NSCLC tumors (25). Justilien has also proven relevant that Ect2 is required for KRAS-Trp53 lung tumorigenesis (26), as well as the fact that PKCι activates NOTCH3 signaling (27). The studies of Justilien and Fields demonstrate that auranofin (a PKCι inhibitor) could be cardinal for treatment (28) and combinations of auranofin with PAK inhibitors deserve further testing (16) (*Figure 1*).

Finally, *KEAP1* mutations are frequent in NSCLC, with *KRAS* mutant NSCLC accounting for 20%. The *KEAP1* gene encodes Kelch-like ECH-associated protein 1, a negative regulator of nuclear factor erythroid 2-like 2 (NFE2L2; NRF2) (29). *KRAS* mutant cell lines carrying *KEAP1* mutations are sensitive to glutaminase inhibition since such cell lines are dependent upon glutaminolysis. Furthermore, NRF2 is a master transcriptional regulator that confers chemoresistance. The clinical outcomes of the SELECT-1 study highlight the limited effect of current therapeutic approaches either with chemotherapy or MEK inhibitors in *KRAS* mutant NSCLC. The Jänne *et al.* study openly shows the dismal outcome of NSCLC patients with *KRAS* mutations and therapeutic solutions should be urgently developed for more molecularly individualized clinical trial models, as is common

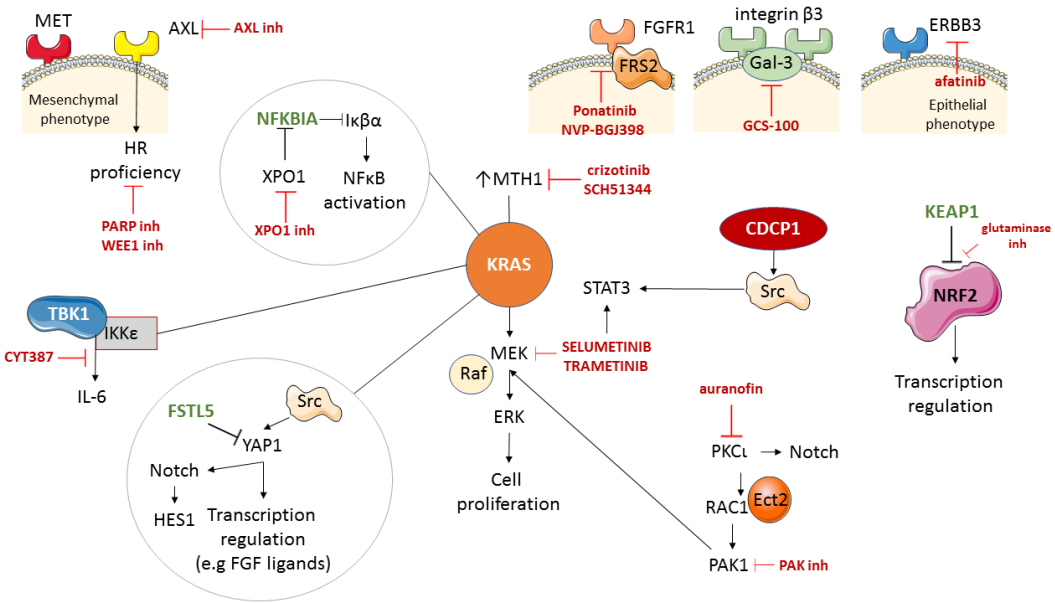

Figure 1 Signaling pathways and regulatory nodes that indicate novel therapeutic approaches for *KRAS* mutant NSCLC.

in breast cancer, such as the My Pathway trial (30). *Figure 1* illustrates several layers of research, including potential biomarkers involving pathways and intercommunication between different components, from RTKs on the cell surface, to the cytoplasm and nuclear components of the tumor cells. Importantly, selective inhibition of MET can lead to overexpression of FRS2 and the combination with FGFR inhibitors is warranted, particularly in mesenchymal tumors displaying elevated expression of AXL. Other opportunities are also depicted in *Figure 1* and the accumulated evidence described herein can help pave the way for better therapies in *KRAS* mutant NSCLC patients.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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