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COMMENTARY

RAS signaling in ALK fusion lung cancer

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ABSTRACT

The success of ALK targeted therapy is blunted by resistance. To identify rational polytherapy strategies to improve clinical outcomes, we studied the molecular basis of ALK oncogene dependence in ALK gene rearrangement positive (ALK+) lung adenocarcinoma. We discovered that RAS-RAF-MEK-ERK signaling is the crucial downstream pathway that is required for ALK+ tumor cell survival. Upfront co-inhibition of ALK and MEK improved response and blocked resistance in preclinical ALK+ lung cancer models, providing rationale for a new treatment paradigm for ALK+ patients.

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There has been a dramatic change in the way that we treat lung cancer in the past decade, in which the use of molecular targeted therapy acting against certain tumor-promoting genetic alterations present in specific subtypes of the disease has resulted in improved patient outcomes.[1] However, targeted therapy resistance remains a barrier to long-term disease control. Dissecting the basis of resistance is important in order to understand the aberrant cell signaling in cancer cells and identify therapeutic strategies to block resistance and enhance patient survival.[1,2]

We recently studied the basis of resistance to targeted therapies acting against the anaplastic lymphoma kinase (ALK) in *ALK* gene rearrangement-positive lung adenocarcinoma (*ALK*+).[3] *ALK*+ disease represents ~5–7%; cases of lung adenocarcinoma, and this subset of patients is currently treated with ALK inhibitors such as crizotinib and ceritinib (among other ALK-targeted agents in clinical trials).[3] Initial ALK inhibitor response is typically incomplete and transient in these patients, with acquired resistance occurring with ~12 months.[4] Additionally, up to 40%; of *ALK*+ patients do not respond to initial ALK inhibitor treatment, showing innate resistance.[4]

In general, efforts to combat resistance have focused on treating acquired resistance after it has already emerged.[2] An alternative strategy to improve initial response and block acquired resistance is to deploy rational upfront polytherapies that target the main oncoprotein (such as oncogenic ALK) and a key downstream effector of that oncoprotein. For instance, upfront (but not second-line) inhibition of $BRAF^{V600E}$ and its main effector, MEK1/2, shows activity that is superior to RAF or MEK inhibitor monotherapy in $BRAF^{V600E}$-mutant melanoma patients.[5,6]

Defining the most appropriate upfront polytherapy strategy is more challenging in cancers with an oncogenic receptor kinase, such as ALK, that can activate several distinct downstream signaling pathways.[7] We addressed this knowledge gap and challenge in *ALK*+ lung adenocarcinoma to identify a rational upfront polytherapy strategy to improve response and patient survival.[7] By studying models of lung adenocarcinoma with the major oncogenic ALK fusion EML4-ALK (echinoderm microtubule associated protein like 4-ALK), we discovered that the RAS-MAPK pathway, but not other established ALK effectors such as PI-3K (phosphoinositide-3 kinase) or JAK/STAT, is required for tumor cell survival. EML4-ALK activated RAS-MAPK (mitogen activated protein kinase) signaling through all 3 major RAS GTPase isoforms (H-, N-, K-RAS), via the HELP (hydrophobic EML protein) domain of EML4. MAPK pathway reactivation via either $KRAS^{WT\ (wild\ type)}$ copy number gain or decreased expression of the MAPK phosphatase DUSP6 promoted ALK inhibitor resistance *in vitro*. Strikingly, these molecular events were each associated with ALK inhibitor resistance in *ALK*+ patients. Combined ALK-MEK inhibition in the upfront setting improved both the magnitude and duration of response in *ALK*+ lung adenocarcinoma preclinical

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models, *in vitro* and *in vivo*. Collectively, our findings uncovered RAS-MAPK dependence as a hallmark feature of *ALK*+ lung adenocarcinoma and indicate that upfront ALK-MEK targeted polytherapy may forestall resistance and improve patient survival.

The findings in our study provide motivation for several new areas of investigation. The data raise the possibility that combining an ALK inhibitor such as crizotinib or ceritinib with a MEK such as trametinib could induce complete tumor response in *ALK*+ patients (Fig. 1). This hypothesis warrants immediate testing in clinical trials. Moreover, $KRAS^{WT}$ copy number and DUSP6 downregulation should be further studied as biomarkers of ALK inhibitor response in *ALK*+ patients, and potentially other tumor types that similarly show MAPK pathway dependence such as those with oncogenic mutations in *RAS-RAF-MEK-ERK* or *EGFR*.

Our findings additionally suggest that the signaling properties of some oncogenic fusion kinases (here, EML4-ALK) may be regulated by specific domains that are present in the fusion partner of the relevant kinase (here, EML4) (Fig. 1). Beyond a role in promoting dimerization of the kinase, our data demonstrate that EML4 (specifically its HELP domain) is crucial both for proper intracellular localization of EML4-ALK and stimulation of RAS and RAF-MEK-ERK signaling. A new layer of regulation of EML4-ALK oncogene function is apparent that is likely to have relevance for the function of other ALK fusion proteins as well as of other kinase fusion proteins more broadly.

The cell biological regulation of the EML4-ALK/RAS interaction and signaling events is an interesting area for future investigation. Determining the precise intracellular localization of EML4-ALK and RAS signaling in cells is crucial, as is defining the molecular complex that links EML4-ALK to RAS GTP loading and downstream signaling. Structure function and high-resolution imaging studies may yield insight and are an active area of focus to fill these knowledge gaps.

Overall, integrated molecular and translational studies will be necessary to more fully define the role and regulation of RAS-MAPK signaling in *ALK*+ cancer cells, and cancer cells with other oncogenic fusion kinases. These studies have important implications for the understanding of the molecular function of RAS and oncogenic ALK in cancer and also for improving patient outcomes.

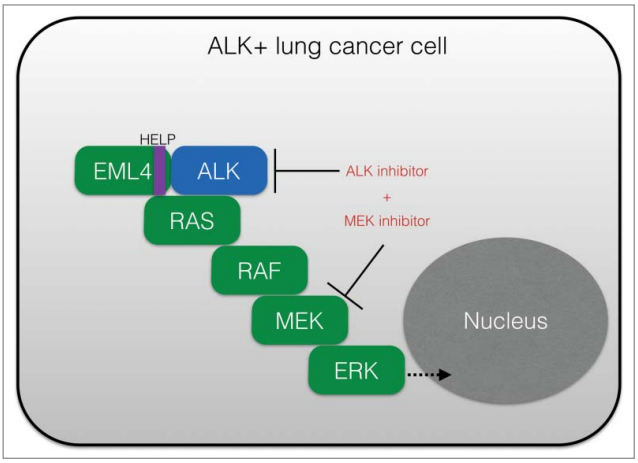

Figure 1. Rational co-targeting of ALK and MEK in *ALK*+ lung adenocarcinoma. Shown is a model of the role of RAS-RAF-MEK-ERK (RAS-MAPK) signaling in *ALK*+ (*EML4-ALK* positive) tumor cells, and the upfront co-targeting strategy to block both ALK and MEK to improve response. MAPK, mitogen activated protein kinase; ALK, anaplastic lymphoma kinase; HELP (hydrophobic EML protein) domain in EML4 (echinoderm microtubule associated protein like 4).

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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