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Targeting the MAPK Pathway in KRAS-Driven Tumors

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KRAS mutations occur in a quarter of all of human cancers, yet no selective drug has been approved to treat these tumors. Despite the recent development of drugs that block $KRAS^{G12C}$, the majority of KRAS oncoproteins remain undruggable. Here, we review recent efforts to validate individual components of the mitogen-activated protein kinase (MAPK) pathway as targets to treat *KRAS*-mutant cancers by comparing genetic information derived from experimental mouse models of KRAS-driven lung and pancreatic tumors with the outcome of selective MAPK inhibitors in clinical trials. We also review the potential of RAF1 as a key target to block *KRAS*-mutant cancers.

The RAS/MAPK Pathway

The RAS/mitogen-activated protein kinase (MAPK) pathway plays a central role in human cancer. It is hyperactivated in a large variety of tumors, and many of its elements have been identified as oncogenes. These observations have generated a profound interest in targeting this pathway as a therapeutic option for cancer (Samatar and Poulikakos, 2014; Yaeger and Corcoran, 2019). The most frequent mutations in this pathway occur in *KRAS*, which are involved in up to 96% of pancreatic ductal adenocarcinomas (PDACs), 52% of colorectal carcinomas, 32% of lung adenocarcinomas, and lower percentages in other tumor types such as endometrial carcinomas (Ryan and Corcoran, 2018). The other *RAS* isoforms are also mutated in human cancer, albeit with lower incidences. In addition to *RAS*, *BRAF* is also mutated in about 8% of all human cancers. These mutations primarily occur in melanomas and at much lower frequency in thyroid, lung, and colorectal cancer. The vast majority of *BRAF* mutations (>90%) generate a constitutively active monomeric protein with high kinase activity that does not require RAS signaling. Other *BRAF* mutations require dimerization but still act independently of upstream signaling. Finally, a third class of mutations that result in low levels or complete inactivation of its kinase activity depend on RAS signaling activated by tyrosine kinase receptors (Yao et al., 2017). *ARAF* or *RAF1* mutations also occur in human cancer but much less frequently. Likewise, mutations in *MEK1* and *MEK2* have been reported to act as oncogenic drivers but are rarely found in human tumors (Gao et al., 2018). Finally, mutations in the *ERK* genes have also been reported, although their implication in oncogenesis has been less well characterized.

RAS proteins activate a plethora of signaling pathways by direct engagement of effector molecules to guanosine triphosphate (GTP)-loaded RAS. The first effector to be discovered was RAF1. Subsequent biochemical analyses demonstrated, along with the related family members BRAF and ARAF, a critical role in transducing signals from membrane-bound, GTP-loaded RAS proteins to MEK and ERK kinases (Malumbres and Barbacid, 2003). Activation of RAF proteins is a complex process involving membrane recruitment via GTP-loaded RAS proteins, followed by a series of phosphorylation and dephosphorylation events leading to conformational changes that culminate in the stimulation of their kinase activity toward the MEK1/2 dual-specificity kinases, which in turn phosphorylate and activate their best known substrates, the ERK family of kinases, ERK1/2 (Terrell and Morrison, 2019). Additional substrates for RAF and MEK kinases outside of the canonical MAPK pathway have also been described (see below; Tang et al., 2015).

RAF activity is also regulated by the formation of homo- and heterodimers as well as by their interaction with scaffold proteins such as KSR1/2 and other regulatory factors such as the 14-3-3 family of proteins (Roskoski, 2010, 2012; Lavoie and Therrien, 2015). Interestingly, homo- and heterodimerization has also been observed within the MEK1/2 proteins as well as between the ERK1/2 isoforms, albeit the full spectrum of mechanistic and functional details of these dimerization events remains to be determined (Santos and Crespo, 2018). At the end of the MAPK cascade, ERK kinases translocate to the nucleus and phosphorylate a large spectrum of substrates, mostly transcription factors that are involved in a variety of processes such as proliferation, survival, and differentiation, in a highly context-dependent manner (Ünal et al., 2017). However, a precise dissection of the contribution of these substrates to ERK signaling either in homeostasis or in tumor development has not been carried out systematically.

Although the MAPK pathway is primarily a linear cascade, its regulation is a highly complex process with feedback loops at every level and a myriad of other factors that participate in fine-tuning the activation or inactivation of each kinase (Kholodenko et al., 2010; Lake et al., 2016). Hence, the MAPK pathway is not only a key element for the oncogenic properties induced by RAS oncoproteins but may also serve as a central reservoir of potential targets to treat RAS-driven tumors (Samatar and Poulikakos, 2014; Ryan et al., 2015).

Attempts to block MAPK signaling in human cancer have yielded mixed results (see below). $BRAF^{V600E}$ inhibitors such as such as vemurafenib and dabrafenib have been shown to be very effective against $BRAF^{V600E}$-mutant melanoma (Savoia et al., 2019). However, no selective drug against any of the other

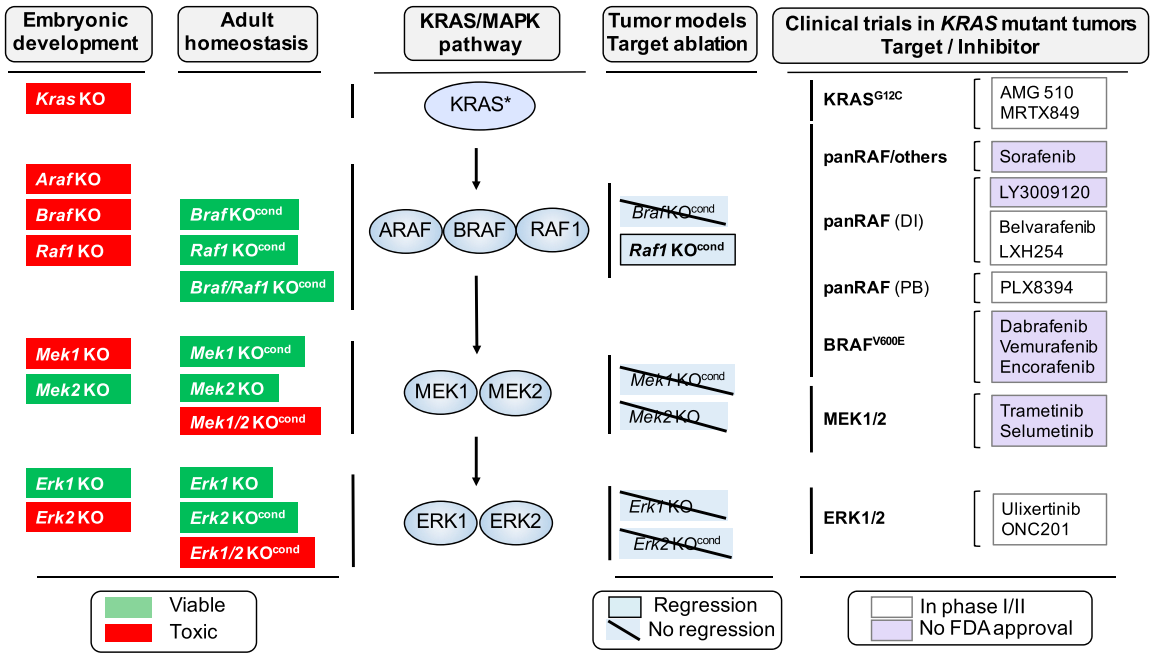

Figure 1. Summary of Validation Studies of Effectors of the KRAS/MAPK Pathway Using Preclinical GEM Tumor Models as well as in Clinical Trials for *KRAS*-Mutant Tumors

(Far left) Embryonic development: effects of germline gene knock-outs in mouse embryonic development. Red boxes indicate embryonic death. Green boxes indicate survival beyond birth. (Second left) Adult homeostasis: effectors systemically eliminated in adult mice. Red boxes indicate high toxicity and rapid death. Green boxes indicate no or acceptable toxicity. KO, knockout; KO^{cond}, conditional knockout. (Center) Schematic diagram of the KRAS/MAPK pathway. (Right) Therapeutic activity upon target ablation in GEM tumor models: crossed-out blue boxes out indicate no significant therapeutic effect upon target ablation. Blue boxes indicate significant therapeutic activity. (Far right) Clinical trials in *KRAS*-mutant tumors: purple boxes indicate inhibitors that failed FDA approval. White boxes indicate inhibitors still in early-phase clinical trials. The primary target(s) for each of the inhibitors is indicated. KO, knockout; KO^{cond}, conditional knockout; DI, dimer inhibitor; PB, paradox breaker.

members of the RAS/MAPK pathway has yet been approved. Although recent developments with selective inhibitors of $KRAS^{G12C}$ oncoproteins in phase I/II clinical trials have caused great excitement (Canon et al., 2019; Hallin et al., 2020), the rest of the oncogenic KRAS isoforms remain undruggable. In this review, we discuss current efforts to identify potential therapeutic strategies against *KRAS*-mutant tumors with particular focus on recent studies suggesting a key role for RAF1 in lung and pancreatic tumors.

Genetic Interrogation of the Role of the MAPK Pathway in Early Development and Adult Homeostasis

Studies using genetically engineered mouse (GEM) models have suggested overlapping as well as unique requirements for the different members of the RAS/MAPK pathway due to either distinct functional activities or to differential patterns of expression (Figure 1). In the case of the RAF proteins, all three isoforms are capable of phosphorylating MEK1/2 in its activation segment. Yet several differences exist when it comes to their role in embryonic development, despite the fact that they seem to be ubiquitously expressed (Wojnowski et al., 2000). Mice deficient for *Raf1* display increased levels of apoptosis in several tissues as well as defects in vascularization and placental development (Hüser et al., 2001; Mikula et al., 2001). Surprisingly, no differences in ERK activation were observed, indicating a high level of functional redundancy among RAF family kinases within the MAPK pathway. More importantly, these studies defined a unique role of RAF1 in protecting embryonic tissues from apoptosis. Mice lacking *Braf* also succumb during embryonic development, in this case due to general growth retardation as well as to its specific requirement in endothelial cells (Wojnowski et al., 1997). The phenotype of *Araf*-deficient mice varies dramatically depending on the genetic background, ranging from neurological and intestinal defects and postnatal death to minor neurological abnormalities with long-term survival (Pritchard et al., 1996). Although this high redundancy complicates the interpretation of the phenotypes observed in individual RAF knockout mice, it has been suggested that BRAF is the main ERK activator *in vivo* (Galabova-Kovacs et al., 2005; Desideri et al., 2015). When systemically deleted in adult mice, ablation of RAF1 or BRAF, either alone or combination, do not seem to induce significant toxicities (Blasco et al., 2011). However, when the three RAF isoforms were ablated in mouse embryonic fibroblasts they immediately ceased proliferation, leading to their eventual death (Drosten et al., 2014).

The role of the two MEK isoforms has also been analyzed genetically. Whereas *Mek2* is dispensable for mouse development and adult homeostasis, *Mek1*-deficient mice die during embryonic development due to placental defects, which can be rescued by tetraploid complementation (Bélanger et al., 2003; Bissonauth et al., 2006; Giroux et al., 1999). Concomitant inactivation of both MEK kinases in the skin of the developing embryo results in embryonic death (Scholl et al., 2007). A specific

kinase-independent role for MEK1 promoting PTEN membrane recruitment has been described (Zmajkovicova et al., 2013). Finally, systemic elimination of *Mek1* and *Mek2* alleles in adult mice causes their rapid death as a consequence of severe intestinal defects, indicating that MEK activity is strictly required for adult homeostasis (Blasco et al., 2011).

Similar phenotypes have been observed in ERK-deficient mice. Whereas *Erk1* is dispensable for embryonic development, disruption of *Erk2* results in embryonic lethality also due to placental abnormalities (Hatano et al., 2003; Nekrasova et al., 2005; Saba-El-Leil et al., 2003). In contrast to *Mek1*-deficient mice, tetraploid complementation experiments fail to yield surviving mice lacking *Erk2*, indicating unique requirements for ERK2 during mammalian development. This is not a consequence of exclusive ERK2 protein functions, since transgenic expression of ERK1 rescues these defects (Hatano et al., 2003; Frémin et al., 2015). Systemic ablation of *Erk1* and *Erk2* in adult mice also causes their rapid death due to toxicities similar to those observed in MEK-deficient mice (Blasco et al., 2011). Taken together, these genetic studies provide evidence for a high level of functional redundancy within each of the three kinase families within the MAPK pathway. Likewise, they underscore the requirement for each of these three main nodes of the MAPK pathway for embryonic development as well as for adult homeostasis (Figure 1).

Role of MEK and ERK Kinases in RAS-Mutant Tumors

The identification of the MAPK cascade as a key effector pathway for RAS oncogenic signaling has generated great interest to block this pathway as a therapeutic strategy for RAS-driven cancers. Genetic studies have helped to dissect the requirements for each of the components of the MAPK pathway especially in KRAS-driven tumors (Figure 1). For instance, individual ablation of *Erk1* or *Erk2* has no effect on initiation of lung adenocarcinomas driven by a resident *Kras* oncogene, yet their combined elimination completely prevents lung tumor formation, indicating that some ERK expression is essential to license lung tumor development. Similar observations were made removing *Mek1* and *Mek2* alleles either individually or in combination (Blasco et al., 2011). At least some MEK expression is also required for RAF1-induced epidermal hyperplasia when eliminated from the epidermis (Scholl et al., 2007). In a skin cancer model driven by DMBA (7,12-dimethylbenz(a)anthracene)/TPA (12-O-tetradecanoylphorbol-13-acetate) treatment, papilloma development is partially inhibited in the absence of MEK1 but not of MEK2 expression (Scholl et al., 2009). Interestingly, selective absence of *Mek1* also causes increased MEK2/ERK or AKT activation through disabled negative feedback mechanisms (Catalanotti et al., 2009; Zmajkovicova et al., 2013). In summary, genetic evidence indicates that whereas complete elimination of MEK or ERK expression efficiently prevents RAS-driven tumors, their systemic elimination causes unacceptable toxicities. Whether inhibition of their kinase activity will cause similar toxic effects is not known. To address this question, it will be necessary to generate inducible knockin strains in which the wild-type proteins, rather than being eliminated, are replaced by kinase-dead isoforms.

Selective Activities of RAF Isoforms in RAS-Driven Tumors

Genetic interrogation of the role of individual RAF isoforms in RAS-driven tumors revealed surprisingly distinct requirements. Whereas BRAF plays a significant role in RAS-driven skin carcinogenesis as the main ERK activator (Kern et al., 2013), it is completely dispensable for the induction of KRAS-driven lung tumors (Blasco et al., 2011; Karreth et al., 2011). On the other hand, RAF1 ablation completely prevents initiation of lung tumor development by resident *Kras* oncogenes (Blasco et al., 2011; Karreth et al., 2011). Likewise, *Raf1* is also required for RAS-driven squamous skin tumor formation (Ehrenreiter et al., 2009). On the other hand, RAF1 expression does not seem to be required for KRAS-driven PDAC initiation (Eser et al., 2013; Blasco et al., 2019). In NRAS-driven melanomas, BRAF exerts specific functions that cannot be compensated by RAF1 during tumor formation. However, in late-stage tumors the activity of both RAF isoforms is required (Dorard et al., 2017). The basis for this differential contribution of RAF isoforms to tumorigenesis in various RAS-driven tumor models is currently unclear. Finally, an unexpected tumor-suppressor role of *Raf1* was reported in hepatocellular carcinoma (Jeric et al., 2016).

Recent studies using a novel "therapeutic" model of aggressive lung adenocarcinoma driven by the concomitant activation of a resident *Kras* oncogene and loss of the p53 tumor suppressor have revealed a unique role for RAF1 expression in tumor maintenance and progression (Sanclemente et al., 2018). Indeed, ablation of *Raf1* from advanced *Kras/Trp53*-driven tumors induces significant rates of tumor regression, including complete disappearance of a limited number of tumors (10%). Tumor regression is likely a consequence of increased rates of apoptosis combined with a reduction in proliferation. Surprisingly, activation of the MAPK pathway does not seem to be affected. Hence, suggesting that the therapeutic consequences observed upon *Raf1* ablation may result from activation of pro-apoptotic pathways, a unique property of this RAF isoform. Importantly, as indicated above, long-term elimination of RAF1 expression in adult mice does not cause significant toxicities, an observation in clear contrast to those results observed upon ablation of MEK1/2 or ERK1/2 expression. These observations raise the possibility that inhibition of RAF1 expression in patients suffering from *KRAS*-driven lung tumors may be a suitable therapeutic option.

As indicated above, *Raf1* is dispensable for development of *Kras/Trp53*-driven pancreatic ductal adenocarcinomas (Eser et al., 2013; Blasco et al., 2019). The reasons for the differential requirements for RAF1 expression between lung and pancreatic tumors remain to be elucidated. However, combined elimination of RAF1 and epidermal growth factor receptor (EGFR) expression, an activity required for the induction of pancreatic metaplasia (Ardito et al., 2012; Navas et al., 2012), completely blocks initiation of tumor development (Blasco et al., 2019). More importantly, combined ablation of *Raf1* and *Egfr* alleles in tumor-bearing mice induced complete tumor regression in a fraction of mice. The precise mechanism by which EGFR ablation cooperates with loss of RAF1 expression remains to be elucidated. Yet, as in the case of lung tumors, ablation of RAF1 either alone or in combination with EGFR has no effect on MAPK signaling (Blasco et al., 2019). Unfortunately, we still do not know why

some tumors respond to this therapeutic strategy whereas others are unaffected. Exomic sequencing did not provide relevant information (Blasco et al., 2019), although RNA-sequencing analysis revealed the differential expression of over 2,000 genes. Hence, the therapeutic effect of the combined RAF1/EGFR ablation is likely to be determined by specific alterations present in each of these pancreatic tumors (Blasco et al., 2019). Of relevance, combined systemic ablation of RAF1 and EGFR did not induce additional toxicities beyond the skin alterations induced by loss of EGFR expression, a toxicity similar to that observed in lung cancer patients treated with EGFR inhibitors (Li and Perez-Soler, 2009).

Specific Functions of RAF1

Although the precise mechanism for the selective requirement for RAF1 in *Kras*-mutant tumors has not been defined as yet, a series of differences exists among the three RAF proteins that could help to explain their differential role in tumor development and/or tumor progression. As described above, all three isoforms can phosphorylate and activate MEK proteins. However, RAF1 possesses a variety of other kinase-dependent and -independent functions that may contribute to its role in cancer. For instance, RAF1 has been shown to phosphorylate RB1 to promote its inactivation and the release of E2F transcription factors (Wang et al., 1998; Kinkade et al., 2008). Moreover, the phosphorylation of MEKK1 and IκB by RAF1 promotes activation of nuclear factor (NF)-κB signaling (Baumann et al., 2000; Li and Sedivy, 1993). It was also demonstrated that BCL2 can relocate RAF1 to the mitochondria where it can phosphorylate and inactivate the pro-apoptotic protein BAD (Wang et al., 1996).

In addition, RAF1 associates with other proteins, modulating their function in a kinase-independent manner. In particular, RAF1 binds to and inhibits the kinase activity of ROCK2 (ROK-α), a kinase known to play multiple roles in cytoskeletal reorganization (Amano et al., 2000; Ehrenreiter et al., 2005). Indeed, it has been demonstrated that this function is selectively required to support RAS-driven squamous tumorigenesis (Ehrenreiter et al., 2009). Hence, ablation of RAF1 expression from established squamous tumors causes their regression via ROK-α-mediated cellular differentiation. It has also been proposed that binding of RAF1 to ROK-α contributes to the anti-apoptotic functions of RAF1, an activity further stimulated by interacting with and blocking the pro-apoptotic activities of ASK1 and MST2 (Chen et al., 2001; O'Neill et al., 2004; Piazzolla et al., 2005). Interestingly, ARAF, but not BRAF, also associates with MST2 in a kinase-independent manner (Rauch et al., 2010). Other kinase-independent functions of RAF1 include its contribution to DNA-damage signaling by binding to CHK2 or to mitosis by associating with PLK1 and AURKA at mitotic spindles (Advani et al., 2015; Mielgo et al., 2011).

Although the full picture of RAF1-mediated activities is constantly being refined, the precise contribution of each of these kinase-dependent or -independent activities of RAF1 in RAS-driven cancers is not completely resolved. For instance, phosphorylation of tyrosine residues 340/341 is believed to be essential for kinase activation, yet mice expressing a RAF1 protein that cannot be phosphorylated at these residues are phenotypically normal. Unfortunately, this mutant protein retains residual kinase activity, thus complicating the interpretation of the results (Barnard et al., 1998; Hüser et al., 2001). A mouse strain expressing a RAF1 kinase-dead isoform due to a D486A mutation within the conserved DFG motif displays a phenotype similar to that of *Raf1* null animals (Noble et al., 2008). However, a D486R mutation, which severely reduces but does not completely inactivates RAF1 kinase activity, is compatible with embryonic development, although mice developed concentric cardiac hypertrophy (Wu et al., 2012). Unfortunately, both mutations also decrease RAF1 stability; thus, the relative contribution of kinase-dependent and -independent functions of RAF1 awaits further clarification. So far, none of these RAF1 mutant isoforms has been interrogated within the context of KRAS-driven tumors.

Pharmacological Inhibition of MAPK Signaling in KRAS-Driven Cancers

Ever since oncogenic drivers were discovered, efforts to develop therapeutic strategies have prioritized the development of selective inhibitors. In the case of *KRAS* oncogenes this strategy has not yielded significant results, since KRAS oncoproteins have been considered undruggable molecules. However, the identification of a small pocket within KRAS along with the possibility of creating a stable covalent bond with a mutant cysteine residue has led to the development of the first selective inhibitors against $KRAS^{G12C}$ oncoproteins (Figure 1). This mutation accounts for 13% of all *KRAS* mutations and is most frequent in lung adenocarcinomas (14%) and colorectal tumors (5%). Early results in phase I/II clinical trials using the $KRAS^{G12C}$ inhibitors AMG 510 or MRTX849 have demonstrated significant responses in about half of the lung patients but not in those suffering from colon tumors (Canon et al., 2019; Hallin et al., 2020). Whether these differential responses are a consequence of the fact that *KRAS* is not a driver in colorectal cancer remains to be determined.

Unfortunately, all other KRAS oncoproteins remain undruggable. Therefore, current strategies to block *KRAS*-mutant tumors have primarily focused on inhibiting downstream MAPK signaling (Figure 1). One of the first drugs aimed at blocking KRAS/MAPK signaling was sorafenib. Although it showed some benefits in a phase II clinical trial for lung cancer, these results were insufficient to merit Food and Drug Administration (FDA) approval to treat this tumor type. Moreover, its limited therapeutic activity was independent of the mutational status of *KRAS* (Kim et al., 2011). Nevertheless this multi-kinase inhibitor, which also possesses activity against RAF kinases, has received approval for the treatment of kidney and liver tumors, possibly due to its effect on other targets (Wilhelm et al., 2006). The identification of *BRAF* mutations in human cancer encouraged the development of inhibitors with higher specificity toward monomeric BRAF mutant proteins such as vemurafenib, dabrafenib or encorafenib (Savoia et al., 2019). These inhibitors have significant therapeutic activity against $BRAF^{V600E}$-driven melanoma, but they have failed to show benefits for patients with KRAS-driven cancer (Durrant and Morrison, 2018). Interestingly, these BRAF inhibitors cause paradoxical ERK activation via promotion of BRAF/RAF1 heterodimer formation and allosteric activation of RAF1. This unexpected effect occasionally results in the development of squamous skin cancer that appears to originate from cells carrying latent *RAS* mutations (Hatzivassiliou et al., 2010; Heidorn et al., 2010; Poulikakos et al., 2010).

In light of these observations, novel RAF inhibitors known as paradox breakers (e.g., PLX8394) have been developed. These inhibitors overcome the paradoxical activation of ERK by disrupting BRAF-containing dimers (Yao et al., 2019; Zhang et al., 2015). These inhibitors show a higher affinity for BRAF homodimers and may be ineffective at blocking RAF1, thus predicting less robust effects on KRAS-driven cancer (Yao et al., 2019). A different class of RAF inhibitors such as LY3009120 preferentially blocks the kinase activity of RAF dimers (Durrant and Morrison, 2018). Unfortunately, preliminary clinical data did not reveal significant benefit for KRAS-driven tumors due to their high toxicity (Ryan and Corcoran, 2018). Additional RAF dimer inhibitors such as belvarafenib and LXH254 are still in early clinical phases. Finally, Athuluri-Divakar et al. (2016) have described rigosertib as a RAS mimetic that blocks the interaction of the RAF and PI3K protein families with KRAS. However, two subsequent papers have proposed that the anti-tumor effect of rigosertib might rather be a consequence of its properties as a microtubule inhibitor (Ritt et al., 2016; Jost et al., 2017). In any case, this inhibitor has been tested in clinical trials for myelodysplastic syndrome but not for *KRAS*-mutant cancers (clinicaltrials.gov NCT02562443). Hence, the precise therapeutic impact of blocking the interaction of the RAF family of proteins with KRAS remains to be determined.

A number of MEK inhibitors have also been developed. Some of these inhibitors, such as trametinib, have already been approved for the treatment of melanoma in combination with selective $BRAF^{V600E}$ inhibitors such as dabrafenib (Yaeger and Corcoran, 2019). Similar results have been obtained combining encorafenib, a $BRAF^{V600E}$ inhibitor, with another MEK inhibitor, binimetinib (Rose, 2019). However, the use of MEK inhibitors such as trametinib and selumetinib as single agents or in combination with chemotherapy has failed to demonstrate significant survival benefits in KRAS-driven lung cancer (Blumenschein et al., 2015; Haura et al., 2010; Infante et al., 2012; Jänne et al., 2017). Finally, clinical development of binimetinib against *NRAS*-mutant tumors was also halted due to unsatisfactory results in phase III trials (Queirolo and Spagnolo, 2017).

Similar negative results were obtained with these MEK inhibitors as single agents or in combination with chemotherapy in pancreatic tumors (Bodoky et al., 2012; Infante et al., 2014). These results are likely a consequence of the toxicities derived from blocking MEK activity, a circumstance that prevents reaching the necessary dosage to achieve anti-tumor responses. Another caveat for the successful clinical use of MEK inhibition in KRAS-driven cancers is the rapid development of resistance. All currently available FDA-approved MEK inhibitors are allosteric non-ATP-competitive inhibitors prone to the rapid development of resistance through a variety of mechanisms that ultimately cause ERK reactivation (Ryan et al., 2015; Savoia et al., 2019). Whether novel MEK inhibitors that do not rely on allosteric sites will tilt the balance between toxicity and anti-tumor effect in favor of the latter remains to be determined.

Currently, several ERK kinase inhibitors have entered clinical trials (Ryan and Corcoran, 2018; Savoia et al., 2019). Two of these inhibitors, ulixertinib and ONC201, have recently moved into phase II (Ryan and Corcoran, 2018). However, neither of them has been selectively tested against *KRAS*-mutant cancers. Since resistance to MEK inhibitors generally involves reactivation of ERK, direct ERK inhibitors may be key for those patients who develop RAF or MEK inhibitor resistance (Ryan et al., 2015). Yet ERK kinase inhibitors may ultimately face shortcomings with toxicity similar to those of MEK inhibitors. Whether inhibitors that target ERK dimerization rather than kinase activity may lead to better clinical outcomes remains to be tested (Herrero et al., 2015). Efforts to identify key ERK substrates in KRAS-driven tumors whose inhibition causes less toxicity may also provide an intriguing alternative.

A series of preclinical studies combining these inhibitors with other drugs have predicted new opportunities for patients with tumors driven by *KRAS* oncogenes (reviewed in Ryan et al., 2015; Ryan and Corcoran, 2018; Samatar and Poulikakos, 2014; Savoia et al., 2019; Yaeger and Corcoran, 2019). Notably, SHP2 or SOS inhibitors have also shown promising activity in combination with MEK or $KRAS^{G12C}$ inhibitors in preclinical studies, indicating that combination therapies are likely to open new doors for the treatment of KRAS-driven tumors (Hillig et al., 2019; Mainardi et al., 2018; Ruess et al., 2018). Indeed, early-phase clinical trials combining the MEK inhibitors trametinib and selumetinib in *KRAS*-mutant lung cancer with other drugs such as erlotinib, lapatinib, and momelotinib have already been initiated (clinicaltrials.gov NCT01229150; NCT02230553; NCT02258607). Moreover, MEK inhibitors are also in early-phase clinical examination in combination with autophagy inhibitors (see, e.g., clinicaltrials.gov NCT04132505), based on highly promising preclinical activity in PDACs as well as other MAPK pathway-dependent tumors (Bryant et al., 2019; Kinsey et al., 2019). Finally, another recent study has demonstrated that an intermittent treatment regimen combining RAF, MEK, and ERK inhibitors may provide effective tumor inhibition while avoiding measurable toxicity in mice (Xue et al., 2017). Hence, the potential of combination treatments for KRAS-driven cancers is just emerging.

Potential Strategies to Inhibit RAF1 in *KRAS*-Mutant Tumors

RAF1 has been recently validated as a relevant therapeutic target in a new generation of mouse models of *Kras/Trp53*-mutant lung and pancreatic cancer in which RAF1 ablation is carried out systemically in tumor-bearing animals. Moreover, systemic *Raf1* ablation in adult mice does not cause major toxicities, as previously reported upon ablation of its downstream MEK1/2 or ERK1/2 kinases (Blasco et al., 2011; Sanclemente et al., 2018). However, any therapeutic strategy directed against RAF1 must be extremely selective to avoid the toxicity derived from interfering with other RAF isoforms. As indicated above, panRAF kinase inhibitors have so far failed in clinical trials due to their high toxicity (Ryan and Corcoran, 2018). Given the high similarities between these proteins, the development of selective RAF1 kinase inhibitors remains a significant challenge.

Fortunately, there are other therapeutic strategies to block RAF1 activity. For instance, it would be possible to block its interaction with the upstream KRAS oncoproteins. However, in this case selectivity might also be as issue. A more selective approach may involve tampering with the interaction of RAF1 with its selective apoptotic effectors such as ROK-α, ASK1,

and MST2, an interaction that does not require its kinase activity (McCormick, 2018). Nevertheless, this strategy will require more robust validation of the potential therapeutic properties of these effectors as well as a more profound knowledge of the overall structure of RAF1, including the identification of those domains involved in its interaction with these potential substrates. Considering the well-known difficulties of blocking protein-protein interactions with small molecules, the possibility to pharmacologically degrade RAF1 appears as a more promising strategy (Gu et al., 2018). Indeed, recent advances in the PROTAC (proteolysis-targeting chimeras) technology may offer opportunities to induce the selective degradation of RAF1 by identifying compounds that could bind to RAF1 within domains that are not present in the other RAF isoforms (Gu et al., 2018). Such PROTAC inhibitors are likely to better recapitulate the therapeutic results observed in GEM models upon *Raf1* ablation (Sanclemente et al., 2018).

Nevertheless, pharmacological targeting of RAF1 in lung adenocarcinomas, either alone or in combination with other inhibitors, as well as in pancreatic tumors in combination with EGFR inhibitors, may not result in widespread therapeutic benefits in human patients. Human tumors carry a larger and more complex mutational load. Moreover, patients are likely to develop resistance, a property that has not yet been investigated in GEM models. In addition, we need to keep in mind that the therapeutic strategies involving RAF1 ablation only induced complete regression in a limited percentage of tumors. Hence, the development of clinically effective therapeutic strategies against *KRAS*-mutant tumors may not only require the development of effective RAF1 inhibitors but also the identification of additional targets in order to expand the spectrum of responding tumors.

In summary, available data from GEM models as well as a limited number of patient-derived xenograft tumors (Sanclemente et al., 2018; Blasco et al., 2019) suggest that blocking RAF1, in combination with other targets, may be a suitable therapeutic strategy for patients suffering from *KRAS*-mutant lung or pancreatic tumors, given not only the observed anti-tumor effects but also the acceptable toxicities resulting from its systemic ablation. In addition, the full spectrum of tumors that require RAF1 expression has not been explored in detail, indicating that other tumor types may also respond to RAF1 inhibition. Carcinogen-induced tumor models may be a way to identify such additional scenarios. On the other hand, this strategy may not be successful in all *KRAS*-mutant tumors, as it appears to occur with $KRAS^{G12C}$ inhibitors that are considerably less effective in colon cancer. In any case, almost four decades after the identification of *KRAS* oncogenes in human tumors, we may start seeing the light at the end of the tunnel. Whether inhibition of RAF1 may contribute to seeing a brighter light is a possibility worth exploring.

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