We recently demonstrated that ablation of RAF1 induces significant levels of tumour regression in mice bearing lung adenocarcinomas induced by KRas/Tp53 mutations. We observed similar results in mice bearing pancreatic tumours providing that RAF1 ablation is combined with elimination of the EGF Receptor. We are now attempting to translate these observations to a pharmacological scenario. Unfortunately, none of the available putative RAF1 inhibitors has shown antitumour activity in the clinic. Therefore, we decided to interrogate by genetic means the best strategy to block RAF1 activity. Unexpectedly, expression of two kinase dead isoforms of RAF1 failed to exhibit therapeutic activity, indicating that RAF1 does not contribute to tumour development via its kinase activity. Hence, pharmacological targeting of RAF1 will require the use of other strategies such as the use of degrons, small chemotypes capable of inducing the degradation of their target proteins.

The KRAS locus encodes 2 protein isoforms, KRAS4A and KRAS4B, which differ in intracellular trafficking and location in the plasma membrane. KRAS mutations in human cancer affect both protein isoforms. Efforts to selectively target the KRAS4B isofrom are under development. We have observed that expression of the endogenous KRAS4A mutant oncoprotein is sufficient to induce lung adenocarcinomas. Hence, effective therapeutic strategies against KRAS mutant tumours must take into account inhibition of both protein isoforms.

“The protein kinase activity of RAF1 is dispensable for KRAS induced tumour development.”

“The oncogenic form of the KRAS4A protein isofrom is sufficient to induce lung adenocarcinomas that undergo proximal metastasis.”
RAF1 kinase activity is not required for KRAS/p53-driven tumour progression

We generated mouse strains that express conditional knockout-in-alleles that encode 2 independent RAF1 kinase dead isoforms, RAF1D468A and RAF1K375M (FIGURE 1A). Surprisingly, systemic expression of these kinase dead isoforms under the control of the endogenous RAF1 locus in mice bearing advanced Kras/Trp53 tumors failed to induce tumour regression (FIGURE 1B). Previous studies indicated that, in addition to its role in MAPK signalling, RAF1 has anti-apoptotic activity. This effect is mediated, at least in part, by its ability to inhibit the pro-apoptotic kinases ASK1 and MST2. Moreover, in vitro studies suggested that this anti-apoptotic activity is likely to be kinase-independent. To interrogate whether the anti-proliferative effect of ablating RAF1 is mediated by these kinases, we blocked proliferation of human A549 lung adenocarcinoma cells as well as of cells obtained from a patient-derived xenograft (PDX) model, PDX-dc1, with lentiviral vectors expressing 2 independent shRNAs against RAF1 (FIGURE 1C). Co-infection of these PDX shRNAs with two independent shRNAs against ASK1 or MST2 restored the proliferative properties of these human lung tumour cells (FIGURE 1D). Re-expression of a cDNA encoding the murine RAF1 protein, whose sequences could not be recognised by the human RAF1 shRNAs, restored proliferation of the A549 and PDX-dct cells to levels similar to those observed upon co-infection with ASK1 or MST2 shRNAs. These results demonstrate that the anti-proliferative effect of silencing RAF1 expression in human lung adenocarcinoma cells is mediated by the pro-apoptotic properties of ASK1 and MST2. These results, taken together, have important implications for the design of effective therapeutic strategies to block progression of KRAS mutant human cancers. They also help to explain, at least in part, the poor results obtained so far in the clinic with RAF inhibitors that either block their kinase or other features that regulate their involvement in MAPK signalling. In summary, these studies strongly suggest that current therapeutic strategies based on inhibition of RAF1 kinase activity are unlikely to produce anti-tumour results obtained so far in the clinic with RAF inhibitors that either block their kinase or other features that regulate their involvement in MAPK signalling. In summary, these studies strongly suggest that current therapeutic strategies based on inhibition of RAF1 kinase activity are unlikely to produce anti-tumour results obtained so far in the clinic with RAF inhibitors that either block their kinase or other features that regulate their involvement in MAPK signalling. In summary, these studies strongly suggest that current therapeutic strategies based on inhibition of RAF1 kinase activity are unlikely to produce anti-tumour results obtained so far in the clinic with RAF inhibitors that either block their kinase or other features that regulate their involvement in MAPK signalling. In summary, these studies strongly suggest that current therapeutic strategies based on inhibition of RAF1 kinase activity are unlikely to produce anti-tumour