Editorial Commentary

PLK1 and its role in the evolving landscape of *KRAS*-mutated colorectal cancer

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Rat sarcoma virus (RAS) consists a family of membrane-bound proteins with GTPase activity. They derive their name from the RAS and initial studies were carried out in the 1960s by Drs. Jennifer Harvey and Werner H. Kirsten, where they induced sarcomas in rodents using a murine leukemogenic virus preparation (1,2). The RAS family of proteins includes three GTPases: Harvey (HRAS), Kristen (KRAS) and neuroblastoma (NRAS) homologues encoded by the corresponding *RAS* genes.

Under normal circumstances, RAS proteins play a central and interconnected role in regulating intracellular signalling, intrinsic to normal physiological cellular proliferation, growth and survival through the RAS-RAF-MEK-ERK mitogen-activated protein kinase (MAPK) signalling pathway (*Figure 1*). Mutations in the RAS-RAF-MEK-ERK pathway genes can result in activation of the pathway leading to unchecked cellular proliferation and oncogenesis. Dysregulation of this pathway occurs very commonly in cancers, with somatic mutations in *KRAS* being found in roughly 30% of all cancers globally, including in lung (30%), colorectal cancer (CRC) (45%), cholangiocarcinoma (15–50%) and pancreatic cancer (>80%) (3). Owing to the complexity and interconnectedness of the pathway, KRAS and other RAS isoforms were long considered to be "undruggable" and have eluded efforts at targeting with inhibitors until very recently.

RAS proteins bind to guanosine diphosphate (GDP) and guanosine triphosphate (GTP) with high affinity. Hydrolysis of GTP to GDP switches RAS from its "active" to its "inactive" state. RAS proteins have an intrinsic GTPase activity, effectively leading them to be self-inactivating. Mutations in *RAS* result in a loss of this intrinsic GTPase activity, leaving the RAS protein in an activated GTP-bound state and persistent stimulation of the downstream signalling cascade. Efforts at targeting RAS have been multimodal, including direct targeting of the mutated RAS protein and effectors in the downstream signalling cascade.

Rationale for targeting KRAS in cancer

The rationale for targeting KRAS in cancer therapy stems from the knowledge of its distinct role in tumorigenesis. Preclinical studies have demonstrated that both primary and metastatic pancreatic cancers depend on sustained KRAS activity (4) and that transgenic mice that express KRAS V12G develop intestinal lesions including invasive adenocarcinoma (5). These findings, combined with the fact that mutations in *KRAS* are seen in approximately 30% of all cancers, make a strong argument for KRAS directed therapy.

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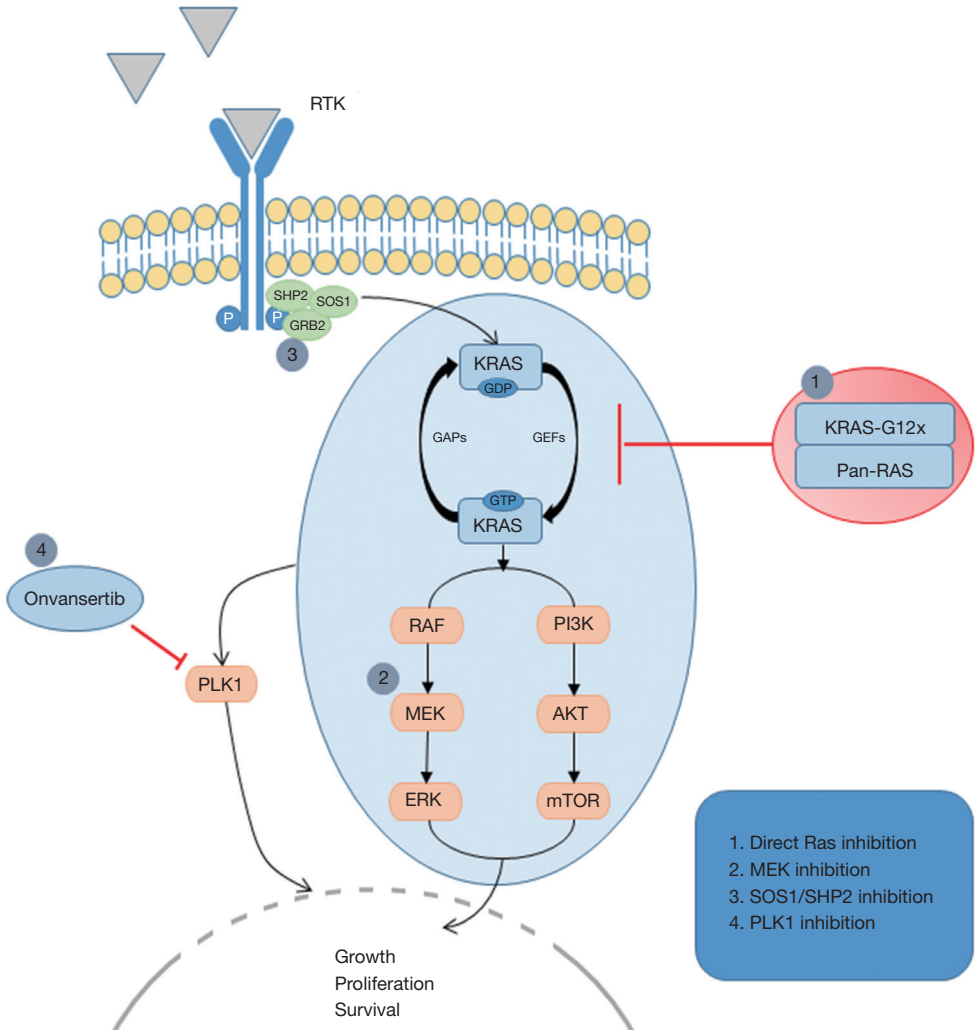

Figure 1 The RAS pathway is a signal transduction cascade initiated by the activation of RTKs on the cell membrane. These RTKs respond to the binding of growth factors. RAS cycles between an active GTP-bound and an inactive GDP-bound state. Stimulation of RTKs leads to the activation of adaptor proteins like SHP2 and SOS1. These adaptor proteins promote the exchange of GDP for GTP on RAS, thereby activating it. Activated RAS then triggers downstream signaling cascades through the MAPK and PI3K pathways. Key points for potential therapeutic inhibition, discussed in the body of the text, are numbered and highlighted in the legend. RTK, receptor tyrosine kinase; GRB2, growth factor receptor bound protein 2; SHP2, Src-homology 2 domain-containing phosphatase 2; SOS1, son of sevenless homolog 1 ; GDP, guanosine diphosphate; GTP, guanosine triphosphate; GAPs, GTPase activating protein; GEFs, guanine nucleotide exchange factors; KRAS, Kirsten rat sarcoma virus; RAF, rapidly activated fibrosarcoma; MEK, mitogen-activated protein kinase kinase; ERK, extracellular signal-regulated kinase; PI3K, phosphoinositide-3 kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin; PLK1, Polo like kinase 1; RAS, rat sarcoma virus.

Drugging KRAS

Over the past 4 decades, significant efforts have been devoted to target RAS. Monotherapies directed at RAS and its downstream effectors have proven to be largely ineffective, due to early adaptive or intrinsic resistance. Combination therapies, targeting two or more critical nodes on the same pathway (vertical pathway inhibition), or those on parallel pathways (horizontal pathway inhibition) are proving to be more effective in overcoming treatment resistance.

Direct inhibition of KRAS

To date, the most successful approaches at inhibiting oncogenic kinases has been the development of small molecule inhibitors that competitively bind to the adenosine triphosphate (ATP) binding domain. Attempts were made to emulate this approach in KRAS inhibition. However, RAS binds GTP rather than ATP at its kinase domain. GTP binding is approximately 1,000-fold tighter than ATP, making this strategy unfeasible. Early efforts at direct inhibition of KRAS led to the development of two small molecule inhibitors SCH53239 and SCH54292. These two molecules were designed to inhibit KRAS by preventing the GDP to GTP transition or binding to the switch II region of the RAS protein, thereby preventing RAS activation. However, due to lack of potency, the development of these compounds was discontinued (6,7).

Inhibition of RAS expression and KRAS processing has also been considered as treatment strategies. Preclinical studies of anti-sense oligonucleotides that inhibit protein synthesis by binding to mRNA at specific sites and inhibiting mRNA translation were very promising; however, these results translated poorly into clinical studies. Although these oligonucleotides were largely well tolerated, they demonstrated minimal clinical activity (8,9). Inhibiting post-translational modifications and activation of KRAS proteins have also been evaluated clinically. RAS proteins are membrane bound. Addition of either a farnesyl or geranylgeranyl moiety to the carboxyl terminus of RAS proteins is a rate-limiting step necessary for the membrane localisation of RAS proteins. Various trials using either farnesyltransferase or geranylgeranyltransferase inhibitors have been conducted. However, these agents demonstrated limited benefit, potentially due to the fact that KRAS can be alternatively prenylated through either the farnesylation or geranylgeranylation pathways conferring resistance to therapy (10,11).

The first real breakthrough in the effort for RAS inhibition is the development of specific KRAS G12C inhibitors such as, sotorasib and adagrasib. As discussed previously, the RAS protein has an inherent GTPase activity, preventing it from becoming constituently active. Although the inherent GTPase activity of RAS is low, it is stimulated by GTPase activating proteins (GAPs) such as neurofibromatosis type 1 (NF1) helping to maintain Ras in its inactive form, thereby preventing persistent activation and uncontrolled cell proliferation. In KRAS G12C mutants, the missense mutation of glycine to cysteine leads to impairment of the GAP mediated hydrolysis of GTP to GDP, resulting in the KRAS protein being locked in a hyper-excited state.

Sotorasib gained approval from the US Food and Drug Administration (FDA) in 2021 as the first targeted agent for KRAS G12C mutated NSCLC. It covalently binds to the mutant cysteine residue at the switch-II-pocket on KRAS, resulting in conformational change and disruption of GTP bound RAS, halting further downstream signalling (12). Sotorasib demonstrated a 48% objective response rate (ORR) and 96% disease control rate (DCR) in the lung cancer cohort of the CodeBreak100 study (13). Results in the CRC cohort were more modest with just a 9.7% ORR and 82% DCR. The increased EGFR signalling in CRC is thought to confer resistance to treatment with single agent KRAS inhibitors and further trials have combined an EGFR inhibitor, such as cetuximab or panitumumab, to a KRAS G12C inhibitor to overcome this resistance mechanism. The Codebreak101 trial combined sotorasib with panitumumab, ORR and DCR improved to 30% and 90% respectively in the CRC cohort (14). Ongoing trials are combining a KRAS G12C inhibitor, an EGFR inhibitor and standard of care chemotherapy for advanced CRC. KRYSTAL-10 phase 3 study is currently examining the combination of adagrasib and cetuximab compared to standard of care second-line therapies with FOLFIRI and mFOLFOX6 in patients with KRAS G12C mutated CRC (15). The INTRINSIC trial is an umbrella study (NCT04929223) examining the combination of divarasib and cetuximab, plus or minus chemotherapy in patients with KRAS G12C mutated CRC.

The KRAS G12C variant accounts for only 3% of CRC cases. Development of inhibitors against more common KRAS variants, such as G12D and G12V, has been complicated by the fact that these mutants have a lower intrinsic GTPase activity than the G12C mutant, thus remaining "active" for longer and the switch II pocket of other variants lacks the reactive cysteine residue found in KRAS G12C mutants (16). Encouragingly, there are multiple agents targeting more common RAS mutants in clinical development, such as MRTX1133 (a non-covalent KRAS G12D inhibitor, NCT05737706) and RMC-6236 (a pan-RAS inhibitor, NCT05379985).

Other targets of RAS inhibition

The RAS pathway is influenced by various affectors, including upstream, downstream and feedback loops. Both

downstream and upstream affectors have been targeted in an attempt to circumvent the lack of efficacy of direct inhibition of RAS. KRAS-mutant tumours are inherently resistant to MEK inhibition secondary to RAF-mediated MEK activation. A vertical combination of KRAS and MEK inhibition with sotorasib and trametinib was evaluated in the Codebreak101 study. Preliminary results are promising with a clinical response noted in 15 of 18 patients in the CRC cohort (17). Another promising combination is the co-inhibition of MEK and CDK4/6. In preclinical studies, tumor regression was seen in 60% of patient-derived xenografts (18). Unfortunately, a randomised phase II trial of MEK and CDK4/6 inhibition versus tipiracil/trifluridine (TAS-102) in metastatic KRAS/NRAS-mutant CRC was halted at interim analysis for futility as binimetinib and palbociclib did not significantly improve median progression-free survival (PFS) or overall survival compared to TAS-102 (19).

The son of sevenless 1 (SOS1) protein and Src homology region 2 domain containing phosphatase 2 (SHP2) represent two potential targets upstream of RAS. SOS1 is a guanine exchange factor (GEF) and activates RAS by promoting the exchange of GDP for GTP. Small molecule inhibitors, agonists and protein degraders have been developed to target SOS1. Preclinical studies have been promising with one study utilising an SOS1 degrader demonstrating 92% SOS1 degradation and growth inhibition in both CRC cell lines and patient-derived organoids. Early phase clinical trials are underway, such as BI1701963 (an SOS1 small molecule inhibitor) in combination with trametinib in solid tumours (NCT04111458) and MRTX0902 (another SOS1 small molecule inhibitor) in combination with adagrasib (NCT05578092).

SHP2 acts as a scaffold, binding to SOS1 and promoting the exchange of GDP for GTP leading to RAS activation. Although SHP2 as a monotherapy has some effect on cell proliferation, preclinical studies demonstrated that co-inhibition of SHP2 and MEK appears synergistic in *KRAS*-mutated disease (3). A number of SHP2 inhibitors, such as TNO155 and RMC4630, are currently in early phase clinical trials.

Targeting Pole like kinase 1 (PLK1)

Inhibition of PLK1 is an interesting new therapeutic option in *KRAS*-mutated CRC. PLK1 is a serine/threonine kinase. It plays a key role in the cell cycle, acting as a conductor of mitosis, and in the DNA damage response mechanism through regulation of the G2-M checkpoint (20). Increased PLK1 signalling in CRC is associated with poorer prognosis. Higher levels of PLK1 are found in CRC tissue compared to normal bowel, and its overexpression is associated with a more aggressive phenotype, increased tumour size and nodal metastasis.

Through an RNA interference (RNAi) screen, it was demonstrated that a synthetically lethal interaction exists between the inhibition of PLK1 and *KRAS*-mutated tumours (21). Ahn *et al.* further explored this mechanism by testing the highly specific PLK1 inhibitor, onvansertib, in both *in-vitro* and *in-vivo* studies. They demonstrated that *KRAS*-mutated cell lines were more sensitive to inhibition of PLK1 than their wild type counterparts. PLK1 inhibition is synergistic with irinotecan in a *KRAS*-mutated CRC mouse xenograft model *in vivo* (22).

These encouraging results from preclinical studies led to this first in human trial of onvansertib co-administered with standard of care chemotherapy. The trial followed a classic 3 + 3 dose escalation design. The recommended phase II dose of 15 mg/m^2 of onvansertib taken orally on days 1 to 5 and days 15 to 19 of a 28-day cycle in combination with FOLFIRI was established. Toxicities were similar to those seen in patients treated with FOLFIRI and bevacizumab in combination, with fatigue, alopecia and gastrointestinal symptoms such as nausea, diarrhea and abdominal pain amongst the most commonly reported adverse events.

A total of 18 patients were enrolled on the study, of which 16 patients were evaluable for response. Six patients had a confirmed response, and 7 demonstrated stable disease, leading to an ORR of 37.5% and a clinical benefit rate of 87.5% in the evaluable population. The median PFS and duration of response was 12.6 months [95% confidence interval (CI): 9.34 months–not reached) and 9.5 months (95% CI: 8.9 months–not reached) respectively. These results compare favourably with previous results in similar patient populations. In the first-line TRIBE trial of FOLFIRI/bevacizumab, the ORR and PFS were 55% and 9.5 months respectively in KRAS-mutant patients (23). In the second-line setting, responses have been far lower, with ORR of 4% and 8% reported in the GERCOR and OPTIMOX1 trials (24,25).

Parallel correlative studies were carried out. Blood samples were collected from patients for circulating tumor DNA (ctDNA) analysis pre-treatment on day 1 cycle 1 and again on day 1 of cycle 2. *KRAS*-mutant ctDNA was detected in 15 of 16 evaluable patients. No difference in baseline *KRAS*-mutant ctDNA levels was seen between patients who had a response

and those that did not. However, a significant decrease in *KRAS*-mutant ctDNA levels was seen in the responders versus non responders after just one cycle of treatment.

Although direct RAS inhibition has shown promise and there are many agents in clinical development, the approach taken by Dr. Ahn and colleagues offers another potential route for RAS inhibition. While we eagerly wait for results from large phase III trials to confirm efficacy of these agents, results from these early phase studies are encouraging. After many years of being considered a non-druggable target, RAS inhibition is finally within reach.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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