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RAS-targeted therapies: is the undruggable drugged?

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Abstract

RAS (*KRAS*, *NRAS* and *HRAS*) is the most frequently mutated gene family in cancers, and, consequently, investigators have sought an effective RAS inhibitor for more than three decades. Even 10 years ago, RAS inhibitors were so elusive that RAS was termed ‘undruggable’. Now, with the success of allele-specific covalent inhibitors against the most frequently mutated version of RAS in non-small-cell lung cancer, $KRAS^{G12C}$, we have the opportunity to evaluate the best therapeutic strategies to treat RAS-driven cancers. Mutation-specific biochemical properties, as well as the tissue of origin, are likely to affect the effectiveness of such treatments. Currently, direct inhibition of mutant RAS through allele-specific inhibitors provides the best therapeutic approach. Therapies that target RAS-activating pathways or RAS effector pathways could be combined with these direct RAS inhibitors, immune checkpoint inhibitors or T cell-targeting approaches to treat *RAS*-mutant tumours. Here we review recent advances in therapies that target mutant RAS proteins and discuss the future challenges of these therapies, including combination strategies.

RAS (*KRAS*, *NRAS* and *HRAS*) is the most frequently mutated gene family in cancers. Mutations in *KRAS* are known drivers of three of the most lethal cancers (lung cancer, colorectal cancer (CRC) and pancreatic cancer). For more than three decades, development of effective therapeutics to inhibit *RAS*-driven oncogenesis has eluded the field and RAS was thought to be ‘undruggable’. However, a clinically approved mutant selective KRAS therapy is now within sight as the FDA has granted an allele-specific covalent inhibitor, AMG 510, Fast Track designation[1]. AMG 510 binds to KRAS-G12C, the RAS mutatant most commonly found in non-small-cell lung tumours. This successful inhibition of KRAS-G12C, has given hope that a range of mutant RAS allele-specific targeted therapies could become therapeutically tractable.

In normal cells, RAS is activated at the membrane downstream of growth factor receptors, including members of the epidermal growth factor receptor (EGFR) family (FIG. 1). This

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Competing interests

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family contains EGFR itself as well as the related ERBB receptors (known as HER in humans). RAS is a small switch signalling GTPase that toggles between its GTP-bound active state and the GDP-bound inactive state. Although RAS proteins exhibit both intrinsic GTP hydrolysis and nucleotide exchange, their cellular signalling state results from activation by guanine exchange factors (GEFs) that catalyse the loading of GTP and deactivation by GTPase-activating proteins (GAPs) that increase hydrolysis of GTP. In its GTP-bound state, RAS directly interacts with and activates several downstream effector pathways including the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathways. Mutations in RAS disrupt the guanine exchange cycle, typically by becoming GAP-independent and 'locking' RAS in the active, GTP-bound state, thereby activating downstream signalling pathways resulting in tumour cell growth.

In this Review, we describe recent advances in the development of therapies targeting mutant RAS proteins and discuss combination strategies that potentially increase the clinical benefit of RAS inhibition (FIG. 1). First, we discuss the prevalence of different RAS isoforms and differences in their biochemical properties. Then, we discuss recent strategies to directly or indirectly inhibit RAS, highlighting the breakthrough therapies that target KRAS-G12C and inhibitors that block Son of Sevenless homologue 1 (SOS1), SHP2 (*PTPN11*) and RAS membrane association. Third, we provide a clinical update on the use of inhibitors targeting RAS itself as well as RAS effector pathways, namely MAPK and PI3K. Last, we detail emerging therapeutic strategies for treating *RAS*-mutant tumours.

RAS mutations and splice variants

RAS mutations are genetic drivers in numerous cancer types including CRC, pancreatic ductal adenocarcinoma (PDAC), lung adenocarcinoma (LUAD; a subtype of non-small-cell lung cancer (NSCLC)), melanoma and certain haematological cancers[2–6]. Although these tumour types are driven by *RAS* mutations, the isoform (*KRAS*, *NRAS* or *HRAS*), codon and frequency of RAS mutations vary by tissue type (FIG. 2). For example, a large percentage of LUAD (32%), PDAC (86%) and CRC (41%) (FIG. 2a) are driven by *KRAS* mutations, which predominantly occur at codon 12 in these tumour types[2,4,5] (FIG. 2b). By contrast, 29% of melanomas are driven by mutations in *NRAS*, and unlike *KRAS*, these mutations occur at codon 61 (REF.[3]) (FIG. 2). *HRAS* mutations occur less frequently than mutations in *KRAS* or *NRAS*, but a subset of head and neck squamous cell carcinoma (HNSCC; 5%) and bladder cancers (6%) are driven by *HRAS* mutations and these mutations occur at either codon 12 or 61 (REFS[7,8]) (FIG. 2a). Genetically engineered mouse models (GEMMs) recapitulate the isoform and codon mutational preferences observed in patient tumours. Expression of $Kras^{G12D}$ in colonic epithelial cells resulted in hyperproliferation, but the expression of $Nras^{G12D}$ in these cells did not alter cell proliferation[9]. Expression of $Nras^{Q61R}$, but not $Nras^{G12D}$, in melanocytes induced melanomas[10]. Approaches to target RAS-driven cancers must therefore account for the specific isoform and the specific codon mutation.

The *KRAS* gene encodes two splice variants which use different exon 4s, resulting in *KRAS4A* and *KRAS4B*. KRAS4A contains an additional 22 or 23 amino acids in the C terminus and therefore has different post-translational modifications and membrane

localization[11–13]. KRAS4B has long been viewed as the major isoform as it is ubiquitously and highly expressed in human cancers[14,15]. Recently, however, *KRAS4A* was shown to be widely expressed in cancer cell lines and expressed at equivalent levels to *KRAS4B* in colorectal tumours[12]. KRAS4A is dispensable in GEMMs: genetic deletion of exon 4A results in viable embryos, whereas deletion of *Kras* results in embryonic lethality[16]. Recently, *Kras4B* was also shown to be dispensable in mice, indicating that the two isoforms are functionally redundant during development[17]. However, deletion of either *Kras4A* or *Kras4B* resulted in resistance to lung tumour formation, suggesting that tumour initiation requires both isoforms[17]. The two isoforms may also have specific roles in the tumour microenvironment — *KRAS4A* expression increases adaptability to stress, such as hypoxia, and *KRAS4B* is expressed in stem cells and progenitor cells. Tumours can adapt, through splicing, to express *KRAS4A* in times of stress[17]. These recent studies have renewed the focus on the role of KRAS4A in tumorigenesis and have shifted the perspective for inhibiting KRAS, as KRAS4A now requires consideration.

Biochemical features of mutant RAS

Small GTPases, such as RAS, cycle between a GDP-bound inactive state and a GTP-bound active state (FIG. 3). GEFs, such as SOS or Ras guanyl nucleotide-releasing protein (RasGRP), promote the exchange of GDP for GTP[18–20]. To stimulate GTP hydrolysis and therefore return RAS to the inactive GDP state, RAS GAPs, such as neurofibromin (NF1) or p120GAP, mediate GTP hydrolysis[21,22]. Mutations in codons 12, 13 and 61 of RAS disrupt GAP-mediated GTP hydrolysis, allowing these mutants to accumulate in a persistently GTP-bound state (FIG. 3a,b). GTP-bound RAS activates downstream effector pathways to promote cell proliferation, most notably the MAPK and PI3K pathways.

Intrinsic GTPase and GDP–GTP exchange rates can vary among the different RAS mutants and this observation may offer insight into how to best target each mutant[23]. For example, codon 12, 13 and 61 mutations generally have diminished intrinsic GTPase activity, except for KRAS-G12C, which exhibits near-wild-type intrinsic GTPase activity despite its reduced p120 GAP-mediated hydrolysis rate[23] (FIG. 3c). Indeed, this unique biochemical property of KRAS-G12C was leveraged by Shokat and colleagues to target KRAS-G12C using covalent inhibitors that bind to the GDP-bound state of KRAS-G12C (REF.[24]). By contrast, KRAS-G13D has elevated intrinsic exchange activity relative to wild-type RAS, suggesting that the GDP-bound state is short-lived[23] (FIG. 3c). *KRAS* with a mutation in codon 13 is partially sensitive to NF1 GAP-mediated hydro lysis, whereas *KRAS* isoforms with mutations in codon 12 or 61 are insensitive to NF1 (REF.[25]). Interestingly, *KRAS* codon 13 mutations are frequently co-mutated with *NF1* mutations, further exemplifying the evolutionary pressure of a tumour to rid KRAS-G13 of NF1 GAP activity and thus become more biochemically active. The biochemical differences between mutant versions of RAS will determine which nucleotide-bound state of RAS would therefore be most appropriate to target with allele-specific inhibitors. Low levels of GTPase activity or high levels of guanine exchange could pose difficulties in targeting the GDP-bound state.

Approaches to target RAS directly

Directly inhibiting RAS is a desirable approach for treating *RAS*-mutant tumours. Here, we highlight the latest efforts in targeting RAS directly, including the development of switch-II mutant-selective covalent inhibitors and pan-RAS inhibitors. Intense efforts in developing mutant-specific RAS (KRAS-G12C) switch-II covalent inhibitors are underway, and progress is being made.

Covalent inhibitors targeting KRAS-G12C

Kras is essential for mouse development, whereas *Nras* and *Hras* are dispensable[26,27]. If humans are similar in this regard, this requirement for *KRAS* creates toxicity concerns when targeting the wild-type KRAS protein. However, when *Kras* is replaced with *Hras*, mice are viable, which reduces toxicity concerns[28]. Conditional deletion of *Kras* in adult mice would directly examine these concerns, but such studies have yet to be published. The field of RAS inhibitors, pioneered by Shokat and colleagues, has focused on covalent inhibition of KRAS-G12C, which would be expected to circumvent toxicity attributed to inhibiting all RAS isoforms.

The inherently reactive nature of cysteine, which is found at codon 12 of KRAS-G12C, can be exploited to create covalent small-molecule inhibitors. Covalently targeting active site cysteines is a widely used strategy in drug discovery[29]. Importantly, wild-type KRAS lacks cysteines in the active site so KRAS-G12C can be specifically inhibited with this covalent approach. As discussed above, the G12 codon is a mutational hotspot in *KRAS* and G12C is the third most common mutation at this position. G12C mutations predominantly occur in LUAD and are transversion mutations (G>T and G>C) associated with smoking (FIG. 2b).

Shokat and colleagues first identified a novel allosteric binding pocket behind switch-II, termed the switch-II pocket, in the mutant KRAS-G12C protein[24] (Figs 4,5a,b). They developed the first series of compounds to irreversibly target KRAS-G12C. These compounds bound KRAS-G12C in the GDP-bound state, blocked SOS-catalysed nucleotide exchange and blocked KRAS-G12C association with RAF[24]. Notably, these compounds only bound to KRAS-G12C in the GDP-bound state and therefore required KRAS-G12C to first undergo GTP hydrolysis. About 75% of KRAS-G12C is GTP-bound in the steady state, but KRAS-G12C has the highest level of intrinsic GTPase activity among the common oncogenic mutations and so is vulnerable to covalent attack[30] (FIG. 3c). Because mutations in *KRAS* other than $KRAS^{G12C}$ have a lower rate of intrinsic GTP hydrolysis, it is unclear whether targeting the switch-II pocket via a similar approach in these other mutant forms will be successful[23].

Numerous companies are developing more potent inhibitors against KRAS-G12C and some of these molecules are in clinical trials, although few details have been published. AMG 510 (FIG. 4) was the first molecule to enter clinical trials and the preliminary results of the phase I trial are promising, particularly in NSCLC: of 13 patients receiving the target dose of 960 mg, 7 patients had a partial response (PR) and 6 had stable disease (SD)[1] (NCT03600883; TABLE 1). The activity in CRC is far less striking: only 1 of 12 patients had a PR and 10 patients had SD[31]. Notably, of the total of 34 patients in the study, none showed dose-

limiting toxicities or adverse events causing discontinuation. In preclinical models, AMG 510 potently inhibited cellular viability exclusively in KRAS-G12C cell lines (half-maximal inhibitory concentration (IC_{50}) ≈ 9 nM and IC_{50} ≈ 6 nM in the cell lines MiaPaCa-2 and NCI-H358, respectively) and induced tumour regression in xenograft models[32]. AMG 510 had synergistic growth inhibitory effects when combined with inhibitors of proteins that activate or are activated by RAS — such as MEK, AKT, PI3K, SHP2 and members of the EGFR family — or with immunotherapy[32].

MRTX849 (FIG. 4) is also in phase I/II clinical trials (NCT03785249; TABLE 1). In early clinical studies (in seven patients at 600 mg twice daily), three of five patients with NSCLC achieved a PR and one of two patients with CRC achieved a PR[33]. In preclinical models, MRTX849 potently reduced cellular viability exclusively in KRAS-G12C cell lines (IC_{50} ≈ 94 nM and IC_{50} ≈ 107 nM in MiaPaCa-2 and NCI-H358 cells, respectively) and caused tumour regression in xenograft models[34]. MRTX849 exhibited synergistic effects, resulting in tumour regression when combined with inhibitors of the EGFR family, SHP2, mTOR, or cyclin-dependent kinase 4 (CDK4) and CDK6, even in MRTX849-refractory tumours[34]. In a CRISPR screen, loss of *NRAS* or *KEAP1* resulted in resistance to MRTX849 whereas loss of *SHP2*, *MYC* or mTOR pathway genes further sensitized tumours to MRTX849 (REF.[34]).

A third KRAS-G12C covalent inhibitor, JNJ-74699157 (ARS-3248), is currently in a phase I clinical trial (NCT04006301; TABLE 1); results have not been published. Two previous compounds (ARS-853 and ARS-1620; FIG. 4) diminished cell growth and inhibited downstream signalling to MAPK exclusively in tumour cell lines with $KRAS^{G12C}$ mutations[30,35,36]. A fourth KRAS-G12C covalent inhibitor, LY3499446, is currently in a phase I/II clinical trial (NCT04165031; TABLES 1,2) as a monotherapy and in combination with inhibitors of CDK4/CDK6 or EGFR or with chemotherapy (docetaxel). Results have not been published.

As the covalent inhibitors discussed require KRAS-G12C to be in the GDP-bound state, resistant mutations could arise in $KRAS^{G12C}$ that disable the GTPase activity or that promote the guanine exchange of GDP for GTP. Proposed resistance mechanisms to covalent KRAS-G12C inhibitors have been identified through CRISPR screens and include the loss of either *NF1* or one of the other RAS isoforms (*NRAS* and *HRAS*)[34,37].

Recently, molecules have been discovered that bind both the GDP-bound and GTP-bound state of KRAS[38]. These molecules bind a new groove (switch-II groove), which is adjacent to the switch-II pocket but away from the nucleotide-binding site (FIG. 5c). This discovery highlights the dynamic nature of the switch-II pocket and, importantly, provides proof-of-concept evidence that both nucleotide-bound states of RAS can be targeted with inhibitors.

Other mutation-specific approaches

As described above, $KRAS^{G12C}$ mutations only account for a proportion of *KRAS* mutations and are primarily found in LUAD (FIG. 2b). To effectively inhibit the other common *KRAS* mutations, $KRAS^{G12D}$ and $KRAS^{G12V}$, different approaches are needed as these mutants lack reactive cysteines in the active site. The development of inhibitors against these specific mutations will need to consider the biochemical differences and evaluate

which state (bound to GDP or GTP) to target, as each mutation will differ in the relative prevalence of these states.

Revolution Medicines is developing a tri-complex platform, RAS(ON), in which a compound mediates a non-natural protein–protein interaction between different mutant KRAS proteins, including KRAS-G12C, and a chaperone, such as cyclophilin A[39]. Formation of such complexes prevents mutant KRAS from binding SOS and RAS-binding domain (RBD)-containing effector proteins. Interestingly, these molecules (RM-007 and RM-008) covalently bind KRAS-G12C and KRAS-G13C, respectively, in the GTP-bound state, and have anti-proliferative activity in cells[39]. As molecules that target the active GTP-bound state break the canonical GTP conformation or block effector interactions, approaches like this, which target the GTP-bound state, would impede the presumed mechanisms of resistance to GDP-bound KRAS-G12C covalent inhibitors.

RAS–effector interaction inhibitors

Although targeting mutation-specific states is effective for KRAS-G12C covalent inhibitors, it will be cumbersome to identify effective therapeutics for each mutant RAS protein. Directly targeting conserved ligand binding sites on all RAS proteins (KRAS4A, KRAS4B, NRAS and HRAS) could provide a single therapeutic approach to inhibit RAS across mutation and tumour types. One such compound, compound 3144, binds a conserved residue, Asp38, in switch-I and blocks RAS effector binding[40]. Compound 3144 bound wild-type KRAS, NRAS and HRAS in vitro and suppressed the growth of KRAS-G13D tumours in vivo; however, toxicity and off-target activity were reported. Indeed, pan-RAS inhibitors are unlikely to be tolerated because RAS is essential in normal cell signalling. Deletion of all three RAS isoforms results in embryonic lethality in mouse models and the absence of cellular proliferation in vitro[41,42].

An early RAS-binding small molecule, DCAI, weakly inhibits SOS1-mediated nucleotide exchange on RAS[43] (FIG. 5d). Cocrystallization with KRAS revealed that DCAI binds a pocket between the α2 helix and the core β-sheet, β1–β3, here referred to as the DCAI pocket, which blocks the interaction between RAS and SOS1. Small molecules that bind in the DCAI pocket prevent SOS1-mediated guanine exchange and so RAS proteins cannot adopt the GTP-bound active state, making this pocket a desirable drug target.

Two independent protein–protein interactor compound series, Abd- and Ch-, reversibly bound RAS in the DCAI pocket and inhibited the interactions of CRAF, RalGDS and the catalytic p110α or p110γ subunits of PI3K with mutant KRAS, NRAS or HRAS[44,45]. A third compound, BI-2852, also bound the DCAI pocket and reduced SOS1-mediated exchange, which reduced the phosphorylation of the downstream kinases ERK and AKT[46].

These series function as pan-RAS inhibitors; the DCAI pocket is present in the wild-type RAS proteins and therefore these compounds are not mutant-selective inhibitors. Further studies are required to optimize parameters for mutant selectivity, as pan-RAS inhibition could pose toxicity issues.

Approaches to target RAS indirectly

Normal RAS activation requires nucleotide exchange, processing, membrane localization and effector binding. Altering one of these essential steps can be used to indirectly inhibit RAS activation.

Inhibitors of the nucleotide exchange cycle

SOS inhibitors.—Initial efforts to block GEF activity towards RAS identified a small molecule that bound KRAS between switch-I and switch-II (K_d = 190 μM), thereby inhibiting SOS binding and SOS-mediated nucleotide exchange[47] (FIG. 5e). The next attempts to inhibit the SOS1–RAS interaction used a molecule designed to mimic an orthosteric SOS helix. Although the compound bound to SOS1 with nanomolar affinity, it had low cellular activity[48,49]. The field then shifted focus and tried to find small-molecule inhibitors of SOS1. Three groups identified small molecules that bound the CDC25 domain of SOS1 and a region adjacent to switch-II on RAS in the RAS–SOS1–RAS ternary complex[50–52]. Interestingly, binding of small molecules to this site could activate or inhibit of the SOS1–RAS interaction[52]. One group identified a small-molecule inhibitor (BAY-293; FIG. 1) that inhibited the SOS1–KRAS interaction at nanomolar levels[52]. However, BAY-293 weakly inhibited *KRAS*-mutant cell proliferation (IC_{50} = 3 μM) but more potently inhibited proliferation of wild-type *KRAS* cells (IC_{50} = 1 μM). Of note, BAY-293 and the KRAS-G12C covalent inhibitor ARS-853 showed synergistic growth-inhibitory effects in a KRAS-G12C cell model. This observation suggests that SOS1 inhibitors could be used in combination with KRAS-G12C inhibitors that bind to the GDP state, as SOS1 inhibition would increase the pool of GDP-bound KRAS-G12C. Currently, a SOS1 inhibitor, BI-1701963, is in a phase I clinical trial as a single agent and in combination with the MEK inhibitor trametinib (NCT04111458; FIG. 1; TABLES 1,2).

SHP2 inhibitors.—SHP2 is a non-receptor protein tyrosine phosphatase that is required for full activation of the MAPK pathway[53]. Mutations in *PTPN11*, which encodes SHP2, cause RASopathies and are found in about 50% of people with Noonan syndrome[54–56]. Although the biological function of SHP2 remains unclear, in the current model SHP2 functions as a scaffold protein, binds GRB2 and SOS1, and thereby increases RAS nucleotide exchange[57–59]. Inhibition of SHP2 would function similarly to a SOS1 inhibitor and block the loading of wild-type RAS with GTP. *KRAS*-mutant tumours depend on SHP2, as deletion of *Ptpn11* in established tumours delays tumour progression, but does not induce tumour regression[60].

SHP-099 (FIG. 1) was found, in a compound library screen for molecules that lock SHP2 in the auto-inhibited conformation, to potently and allosterically inhibit SHP2 (IC_{50} = 0.071 μM)[61]. SHP-099 synergistically reduces cell proliferation when combined with trametinib in KRAS-G12D patient-derived organoids and xenograft models of PDAC and NSCLC[60,62]. However, no sensitivity to SHP-099 was observed in a KRAS-G13D-mutant cell line, MDA-MB-213 (REF.[61]).

A potent and selective SHP2 allosteric inhibitor, RMC-4550 (FIG. 1), binds at the same site as SHP-099 and stabilizes the auto-inhibited conformation of SHP2 (REF.[63]). Treatment

with RMC-4550 reduced cell proliferation in preclinical models but this effect was evident only in cells harbouring mutations in codon 12, not codon 13 or 61, of *KRAS*. Moreover, the greatest sensitivity was observed in *KRAS*^{G12C}-mutant cells; *KRAS*^{G12D}- or *KRAS*^{G12V}-mutant cells were modestly sensitive to the compound. This observation highlights that the biochemical properties of each mutation determine the extent to which RAS activity depends on guanine exchange, and that mutations with elevated intrinsic or GAP-mediated hydrolysis are particularly sensitive to SHP2 inhibition.

The clinical candidate derived from RMC-4550, RMC-4630 (FIG. 1), is currently in a phase I monotherapy clinical trial (NCT03634982; TABLE 1) and a phase Ib/II clinical trial in combination with another MEK inhibitor, cobimetinib (NCT03989115; TABLE 2). A second SHP2 allosteric inhibitor, JAB-3068 (FIG. 1), is currently in a phase I/II clinical trial and results have not been published (NCT03518554, NCT03565003; TABLE 1). A third SHP2 inhibitor, TNO155 (FIG. 1), is in a phase I monotherapy clinical trial (NCT03114319; TABLE 1) and a phase I/II clinical trial in combination with MRTX849 (REF.[64]) (NCT04330664; TABLE 2). Results from these studies have not been published.

Inhibitors of RAS processing

RAS is only active when localized to the cell membrane. To associate with the membrane, RAS requires three enzymatic post-translational processing steps: prenylation of the CAAX box by farnesyltransferase (FTase) or geranylgeranyltransferase (GGTase); cleavage of the terminal AAX residues by RAS-converting enzyme (RCE1); and methylation of the cysteine residue of the CAAX box by isoprenylcysteine carboxyl methyltransferase (ICMT). Inhibition of RAS processing should prevent membrane association and downstream RAS signalling.

Although FTase inhibitors (FTIs) were clinically disappointing in *KRAS*-mutant cancers, possibly because of the functional redundancy between FTase and GGTase, there is a renewed focus on the use of FTIs in *HRAS*-mutant cancers. Unlike KRAS and NRAS, HRAS is prenylated exclusively by FTase so FTIs could be useful for treating *HRAS*-mutant cancers[65]. Indeed, responses to the FTI tipifarnib were observed in patient-derived models of *HRAS*-mutant HNSCC and NSCLC[66]. Tipifarnib is currently in a phase II clinical trial for the treatment of *HRAS*-mutant HNSCC and thyroid cancer (NCT02383927; TABLE 1). In the reported results, six patients received tipifarnib of whom four had a PR and two had SD[67].

A strategy to circumvent the compensation of FTIs by GGTase in *KRAS*-mutant and *NRAS*-mutant tumours is to target the downstream RAS processing enzymes, RCE1 and ICMT, which are essential in *KRAS*-mutant cells, but not wild-type *KRAS* cells[68]. By contrast, the β-subunit of FTase is essential in cells with either wild-type or mutant KRAS, so the complete loss of FTase activity is detrimental to all cells[68]. Inhibition of RCE1 or ICMT may provide mutant selectivity and reduce the toxicity with FTIs.

A small-molecule inhibitor of ICMT, cysmethynil (IC_{50} = 2.4 μM), impaired RAS membrane association and reduced cellular growth of *RAS*-mutant cell lines in vitro (half-maximal effective concentration (EC_{50}) ≈ 20 μM) and in vivo[69–71]. Structural modification

of cysmethynil improved the potency of ICMT inhibition but this compound did not inhibit cellular growth[72]. The most potent ICMT inhibitor to date (IC_{50} = 1.3 nM) only mildly reduces proliferation (EC_{50} = 0.3 to>10 μM)[72]. Recently, another ICMT inhibitor, UCM-1336 (IC_{50} = 2 μM), was found to impair membrane association of all four RAS isoforms, regardless of mutational status, and to reduce the cellular growth of *RAS*-mutant cell lines in vitro (EC_{50} = 2–12 μM) and in vivo[73]. UCM-1336 is structurally distinct from the cysmethynil-derived compounds, but still requires further enhancement for effective therapeutic use. No potent and selective inhibitors have been identified for RCE1 (REFS[74,75]).

After post-translational modification, RAS localization and trafficking is regulated by the prenyl-binding protein phosphodiesterase-δ (PDEδ), which binds to farnesylated RAS[76]. Deltarasin, a small molecule that binds to the farnesyl-binding pocket of PDEδ, prevents KRAS binding (K_d = 7.6 nM) and results in mislocalization of KRAS and reduced cell proliferation[77]. Computational docking later identified another compound, NHTD, which disrupts KRAS binding to PDEδ[78]. NHTD inhibited the growth of mutant KRAS tumour cells in vitro and in vivo (EC_{50} = 2–7 μM), but did not have the cytotoxic effects associated with deltarasin.

Notably, the enzymes discussed above also process other membrane-associated proteins, which could lead to off-target effects. Direct inhibition of RAS therefore has the most potential as a primary therapeutic option.

RAS oligomerization and effector binding

Once RAS is effectively processed, RAS proteins localize in the membrane where they undergo oligomerization or dimerization, which is required for effective RAS-driven signalling. RAS proteins were observed to organize into isoform-specific assemblies of five to nine proteins termed ‘nanoclusters’, which are important for the activation of downstream pathways[79–85]. More recently, RAS dimerization has emerged as another possible mechanism by which RAS can self-associate to enhance scaffolding and signalling activities[86–89].

RAS dimers and oligomers are difficult to reconstitute in vitro and typically must be studied in the context of post-translationally modified RAS within reconstituted lipid membranes or nanodiscs. Owing to the absence of definitive structural and biochemical data, most efforts to define the molecular details of RAS–RAS interaction surfaces have been restricted to computational modelling combined with experimental validations, as well as leveraging information from crystal packing interactions across the hundreds of RAS structures deposited in the Protein Data Bank (PDB; see Related links)[88,90–93]. Although numerous interfaces have been identified and validated, many computational and experimental studies have converged on a single RAS dimerization interface, the α4–α5 interface, which is also prevalent as a crystal packing interaction in many of the HRAS, KRAS and NRAS structures.

The proposed α4–α5 interface can be targeted with a nanobody, NS1 (REF.[92]) (FIG. 5f). NS1 disrupts HRAS and KRAS self-association through directly binding the α4–α5

interface (K_d = 13 nM for HRAS and 65 nM for KRAS), reducing activation of downstream pathways and inhibiting cell proliferation, while leaving RAS localization and GTPase activity unperturbed[92,94]. Further work should define and validate these RAS–RAS interaction surfaces: a recent study demonstrated that fully processed KRAS remains monomeric in lipid membranes across a wide range of concentrations[78].

A newly discovered mechanism of RAS auto-inhibition, membrane occlusion, has provided another potential avenue for targeting RAS protein–protein interactions. In membrane occlusion, direct interactions between RAS and the lipid membrane sequester the effector binding interface of RAS away from the cytosol[95,96]. A small molecule, Cmpd2, can bind to the interface between RAS and the lipid membrane (K_d = 1 μM), promoting membrane occlusion and reducing binding to the RBD domain of RAF[95]. Owing to the highly conserved nature of the RAS–effector interface (FIG. 5g), this type of strategy provides an opportunity to effectively inhibit all downstream signalling pathways driven by RAS.

Targeting the RAS pathways

Two distinct approaches exist to target the RAS pathways: identifying genes that are synthetically lethal with *RAS* mutations, or targeting the tyrosine kinase receptors (EGFR family) and RAS effector pathways, namely MAPK and PI3K.

Synthetic lethal screens.—The Broad Institute dependency map (DepMap) portal compiles gene essentiality scores from CRISPR screens in hundreds of cancer cell lines (see Related links). In *KRAS*-mutant and *NRAS*-mutant cell lines, besides *RAS* itself, the top essential genes are *RAF1* (which encodes CRAF) and *SHOC2*. An independent study showed that *RAF1* and *SHOC2* are required in *RAS*-mutant human acute myeloid leukaemia cell lines[68].

Ablation of *Craf* in a *Kras*^{G12V}*;Trp53*$^{-/-}$ LUAD mouse model significantly reduced tumour size[97]. The authors observed no adverse systemic effects of CRAF ablation and the loss of CRAF did not alter MAPK signalling, suggesting that kinase-independent CRAF functions may play a role in this context. Furthermore, combined ablation of *Egfr* and *Craf* in *Kras*^{G12V}*;Trp53*$^{-/-}$ PDAC mouse models induced complete regression of PDAC tumours[98]. Again, the dual ablation of *Craf* and *Egfr* was well tolerated in the GEMMs. In a second approach, using PDAC patient-derived xenografts (PDXs), the dual knockdown of *EGFR* and *CRAF* expression also reduced tumour growth. These findings highlight the therapeutic advantage of inhibiting CRAF and suggest the need for selective inhibitors against CRAF. However, novel approaches are likely to be required for selective CRAF inhibition because of the high homology between RAF family kinase domains and the fact that CRAF may have a kinase-independent function in this context.

A phosphatase complex containing SHOC2, MRAS and protein phosphatase 1 (PP1) dephosphorylates RAF at Ser259. This dephosphorylation alleviates the negative regulation of RAF by 14-3-3 and enables RAF dimerization[99]. Therefore, SHOC2 promotes RAF dimerization and, importantly, SHOC2 is required for maximal ERK activity. Ablation of *Shoc2* in a *Kras*^{G12D}*;Trp53*^{R172H} LUAD mouse model reduced tumour burden and pro longed survival without toxicity[100]. In a second model, genetic deletion of *SHOC2* in

KRAS-mutant and *EGFR*-mutant NSCLC cell lines reduced growth in xenograft models and sensitized cells to MEK inhibition (using selumetinib, trametinib, PD0325901 or pimasertib). A CRISPR–Cas9 screen identified that SHOC2 loss is synthetically lethal with trametinib treatment[101]. These findings suggest that SHOC2 inhibitors could not only provide therapeutic benefit as a single agents, but potentially also in combination with MEK inhibitors.

EGFR family therapies.—There is also evidence of interplay between RAS and the EGFR family of receptor tyrosine kinases. As the EGFR family is upstream of RAS, inactivation of these receptor tyrosine kinases can reduce RAS activation[36]. Until recently, studies have focused on inhibition of EGFR in particular for the treatment of *RAS*-mutant tumours.

The use of cetuximab or panitumumab, monoclonal antibodies against EGFR, improved overall and progression-free survival in patients with metastatic CRC and wild-type *KRAS* alleles (also with no mutations in *NRAS*, *BRAF* or *PI3KCA*, which encodes the catalytic subunit of PI3Kα), but these antibodies had no effect in *KRAS*-mutant tumours, specifically those with mutations at codons 12 or 13 (REFS[102,103]). The results of these clinical trials led to the approval of the use of cetuximab and panitumumab in the treatment of metastatic CRCs that lack mutations in *KRAS*. Additionally, EGFR tyrosine kinase inhibitors (TKIs), such as erlotinib and gefitinib, which are approved for the treatment of *EGFR*-mutant NSCLC, are ineffective as a monotherapy in *KRAS*-mutant NSCLC[104–107]. Erlotinib is approved in combination with gemcitabine for the treatment of PDAC, albeit with limited benefit: over 90% of patients with PDAC have *KRAS* mutations[108].

A retrospective study of the clinical trials of cetuximab and panitumumab in metastatic CRC analysed their clinical benefit in relation to the specific codon mutation. Patients with KRAS-G13D-mutated tumours ($n = 32$) had increased overall survival and progression-free survival compared with those with other *KRAS* mutations when receiving cetuximab[109]. However, in a second retrospective analysis of panitumumab, patients with KRAS-G13D-mutant tumours were deemed unlikely to benefit from this treatment[110]. A phase II clinical trial, ICECREAM, is underway to evaluate the efficacy of cetuximab treatment in patients with metastatic CRC with $KRAS^{G13D}$ mutations (ACTRN12612000901808; TABLE 2)[111].

Although wild-type *KRAS* CRCs respond to cetuximab treatment, ultimately metastatic CRCs become refractory to anti-EGFR treatment and acquire *KRAS* mutations as a mechanism of resistance. In one study, these mutations included *KRAS* amplification (1 of 11) or *KRAS* somatic mutations, most commonly G13D (5 of 11)[112]. However, it is unclear whether these mutations were present prior to cetuximab treatment in a *KRAS*-mutant clone, or arose as a result of treatment.

Genetic depletion of *Egfr* suppresses the development of $Kras^{G12D}$-driven PDAC and NSCLC in GEMMs[113–115]. Upregulation of EGFR family members other than EGFR itself, such as ERBB2, ERBB3 and ERBB4, was found as an early event in tumours with either KRAS-G12D or erlotinib resistance[115,116]. Interestingly, patients with NSCLC, including those with mutations in *EGFR* or amplification of *EGFR*, *HER2*, *HER3* or *HER4*, had poor

survival[117]. Treatment of autochthonous KRAS-G12D tumours with the FDA-approved pan-ERBB inhibitors, afatinib or neratinib, reduced both tumour size and initiation compared with erlotinib or gefitinib treatment[115,116]. Currently, a phase I/II clinical trial is evaluating the efficacy of neratinib in *RAS*-mutant solid tumours in combination with the histone deacetylase inhibitor divalproex sodium[118] (NCT03919292; TABLE 2).

Pan-ERBB and EGFR inhibitors have promise in combination with MEK or KRAS-G12C covalent inhibitors. ERBB3 suppression with afatinib sensitized *KRAS*-mutant CRC and NSCLC cell lines to MEK inhibition (by selumetinib)[119]. Afatinib or neratinib treatment, in combination with MEK inhibition (by trametinib), increased the survival of KRAS-G12D NSCLC mouse models[116]. The animals were treated daily for one week and monitored for survival, indicating that this combinatorial approach induces a durable response. Treatment of KRAS-G12C cells with ARS-853 and either erlotinib or gefitinib gave a synergistic effect and erlotinib decreased the fraction of KRAS-G12C bound to GTP[35,36]. As ARS-853 and other KRAS-G12C covalent inhibitors bind to KRAS-G12C in the GDP-bound state, the efficacy of EGFR TKIs suggests that EGFR is capable of activating mutant RAS and the inhibition of EGFR reduces the amount of GTP-bound KRAS-G12C, providing increased efficacy.

MAPK pathway: RAF inhibitors.—Active GTP-bound RAS promotes RAF dimerization and phosphorylation, which induces RAF kinase activity and results in phosphorylation of the RAF substrates MEK1 and MEK2. The phosphorylation cascade continues with MEK phosphorylation of the terminal kinases, ERK1 and ERK2. ERK kinase activity both activates growth-promoting transcription factors, including members of the ETS family, and participates in negative feedback loops[120]. This dynamic process controls the duration and amplitude of the MAPK signalling cascade. ERK can negatively suppress MAPK signalling by phosphorylating upstream components, SOS or CRAF, or by altering the transcription of the dual-specific phosphatase (DUSP) family and the Sprouty (SPRY) family[121–124]. DUSPs dephosphorylate ERK and SPRYs inhibit receptor tyrosine kinase signalling by sequestering SOS–GRB2 (REFS[125,126]). To effectively treat *RAS*-mutant tumours, the MAPK pathway must be almost completely suppressed. One strategy to achieve this is to use kinase inhibitors against components of the MAPK pathway, in combination with therapies that target other mechanisms discussed in this Review.

Currently, kinase inhibitors targeting BRAF-V600 and MEK are approved for *BRAF*^{V600}-mutant metastatic melanoma but not for *RAS*-mutant tumours. Clinically approved BRAF-V600 inhibitors, such as vemurafenib and dabrafenib, induce an outward shift of the αC helix in the kinase domain of RAF[127]. These inhibitors effectively inhibit RAF monomers (BRAF-V600 signals as a monomer) but cannot be used for *RAS*-mutant tumours, which signal through BRAF and CRAF dimers. Furthermore, in *RAS*-mutant tumours, these inhibitors have been shown to paradoxically activate the MAPK pathway by binding to wild-type RAF, inducing RAF dimerization and the downstream phosphorylation of MEK and ERK[128,129]. Paradoxical activation depends on the mode of inhibitor binding: RAF dimer inhibitors exhibit far less paradoxical activation than approved BRAF-V600 inhibitors in *RAS*-mutant tumours[130].

RAF dimer inhibitors, such as AZ-628, belvarafenib, LY3009120 and LXH-254, bind RAF in a DFG-motif 'out', αC helix 'in' active position[131–133] (FIG. 1). These inhibitors are effective against RAF monomers and dimers, and although they can also promote dimerization, they have minimal paradoxical activation, as they can bind both dimer components. Many of these inhibitors act as pan-RAF inhibitors and have been reported to have efficacy in *RAS*-mutant and *BRAF*-mutant tumours[131,132,134–139]. A phase I clinical trial evaluating LY3009120 was terminated owing to lack of clinical efficacy as the best overall outcome was SD (observed in eight patients (15%))[140].

Currently, two pan-RAF inhibitors are under phase I clinical evaluation for the treatment of *RAS*-mutant tumours: belvarafenib (NCT02405065, NCT03118817; TABLE 1) and LXH-254 (NCT02607813; TABLE 1). Belvarafenib has clinical activity as a monotherapy in subsets of patients with *BRAF*-mutant and *NRAS*-mutant tumours, with a PR observed in two of six individuals with *BRAF*-mutant melanoma, two of seven with *BRAF*-mutant CRC and two of nine with *NRAS*-mutant melanoma[138]. Treatment with belvarafenib, unlike BRAF-V600 inhibitors, did not induce squamous cell carcinomas, a known result of paradoxical activation[138,141,142].

MAPK pathway: MEK inhibitors.—Currently, there are three allosteric kinase inhibitors targeting MEK — cobimetinib, trametinib and binimetinib — clinically approved for the treatment of $BRAF^{V600}$-mutant melanoma. Clinical trials evaluating MEK inhibitors as monotherapies for *RAS*-mutant tumours have found no improvement and none of the MEK inhibitors are clinically approved for treating *RAS*-mutant tumours[143–147]. MEK inhibitors, like RAF inhibitors, induce pathway feedback loops in *RAS*-mutant tumours, resulting in relatively modest efficacy in these tumours[123,148–150].

Although single-agent MEK inhibitors have largely failed in the clinic for *RAS*-mutant tumours, *NRAS*-mutant melanomas have some sensitivity[151,152] (FIG. 1). For example, in a phase II trial of binimetinib, 20% of people with *NRAS*-mutant melanomas had a PR[151]. Based on these data, a phase III trial of binimetinib compared with dacarbazine is ongoing to evaluate the efficacy in treating patients with $NRAS^{Q61}$ mutations (NCT01763164; TABLE 1). Additionally, in a phase II trial, pimasertib improved progression-free survival compared with dacarbazine in patients with *NRAS*-mutant melanoma (NCT01693068)[152]. In the overall patient population, pimasertib failed to improve overall survival compared with dacarbazine and did not receive FDA approval[153]. Notably, similar clinical trials of MEK inhibitors for *KRAS*-mutant PDAC, CRC and NSCLC have shown no benefit over the standard of care[147,154–156]. In cell culture, *NRAS*-mutant melanoma cells have greater sensitivity to pan-RAF inhibition than *KRAS*-mutant cells and have comparable sensitivity to $BRAF^{V600E}$ cells[130]. The differential sensitivity for *NRAS*-mutant melanoma requires further exploration to determine whether the sensitivity is due to an intrinsic property of melanoma, mutant *NRAS* or the biochemical properties of codon 61 mutations.

As MEK or RAF inhibitor monotherapy failed to provide clinical benefit for *KRAS*-mutant tumours, the efficacy of these inhibitors used in combination is being explored in the clinic. Owing to the complexity of the feedback loops within the MAPK pathway, targeting multiple nodes of the pathway could lead to sustained and durable suppression of

phosphorylated ERK (a measure of activated ERK and a direct target of MEK; phosphorylated ERK is commonly used to measure overall MAPK pathway activity). Indeed, the combination of MEK and RAF inhibitors exhibited synergy in preclinical models of *RAS*-mutant tumour cells and blocked pathway reactivation[130]. Furthermore, the induction of phosphorylated MEK and GTP-bound RAS upon MEK inhibitor treatment is required for the synergistic effect of MEK and RAF combination treatment[130]. The strongest synergistic effects of MEK and RAF inhibition were observed in cells with mutant KRAS with high levels of intrinsic nucleotide exchange, such as KRAS-G13D, which enhances GTP-bound RAS levels: this observation suggests that RAF dimerization, induced by MEK inhibition, probably contributes to this synergy. Currently, two phase I clinical trials combining RAF and MEK inhibitors are underway. One is investigating the combination of belvarafenib and cobimetinib (NCT03284502; TABLE 2) and the other is investigating the combination of trametinib and LXH-254 (NCT02974725; TABLE 2).

MAPK pathway: ERK inhibitors.—Inhibition of ERK, the culminating kinase in the cascade, could directly reduce the oncogenic transcriptional output and provide a valuable therapeutic option for tumours that are resistant to MEK or RAF inhibition[157,158]. The clinical development of ERK inhibitors lags behind the development of MEK, BRAF monomer and pan-RAF inhibitors. In addition, the early clinical trials of ERK inhibitors for the treatment of *RAS*-mutant tumours have been largely unsuccessful (FIG. 1).

The preclinical compound SCH-772984 (FIG. 1) acts as a dual mechanism ERK inhibitor by binding to and inhibiting ERK1/2 while also inducing a conformational shift that prevents ERK1/2 phosphorylation by upstream kinases[159,160]. Treatment of *RAS*-mutant cancer cell lines with SCH-772984 reduced levels of phosphorylated ERK and reduced cell proliferation[161]. The clinical compound, MK-8353, was developed by Merck to have improved pharmacokinetic properties compared with SCH-772984 (FIG. 1). Similar to SCH-772984, MK-8353 acts as a dual mechanism inhibitor and reduced cell proliferation of *NRAS*-mutant melanoma cell lines in preclinical models[160,162]. However, in a phase I monotherapy clinical trial for MK-8353, no antitumour responses were observed amongst the 26 patients with *KRAS* or *NRAS* mutations who were enrolled[160]. Three PRs, however, were observed in patients with $BRAF^{V600}$-mutant melanoma. MK-8353 is under clinical evaluation in combination with selumetinib (NCT03745989; TABLE 2) and pembrolizumab (NCT02972034; TABLE 2) in trials that include patients with *RAS*-mutant tumours.

GDC-0994 (an ERK inhibitor, FIG. 1) exhibited efficacy in combination with cobimetinib in KRAS-mutant tumour models[163]. Although preclinical xenograft models showed a therapeutic dose leading to growth reduction could be achieved, the phase I clinical trial of cobimetinib and GDC-0994 was terminated because patients could not tolerate the combination[163,164].

However, when GDC-0994 was evaluated as a monotherapy, in a phase I clinical trial, the recommended phase II dose of 400 mg daily on a 21-days-on/7-days-off schedule was tolerable[165]. In the trial, 14 patients with *BRAF*-mutant CRC or gastric cancer were treated with GDC-0994 and two patients had a PR, seven had SD and five had disease progression[165]. Of the 14 patients enrolled with *KRAS*-mutant tumours, four achieved SD

and ten had progressive disease[165]. GDC-0994 induced MAPK pathway suppression (19–51%) in paired tumour biopsies using NanoString gene expression, and greater suppression was observed in patients with *BRAF*-mutant CRC (three of four) than in those with *KRAS*-mutant PDAC (one of four)[165]. Further evaluation of *NRAS*-mutant tumours is required in a phase II study as only one patient was evaluated at the lower dose of 100 mg daily[165].

In a recently completed phase I clinical trial, ulixertinib (BVD-523; FIG. 1) showed antitumour effects in *NRAS*-mutant melanoma and *BRAF*-mutant solid tumours[166]. Ulixertinib is a selective, reversible, ATP-competitive ERK1/2 inhibitor[166]. In the trial, 17 patients with *NRAS*-mutant melanoma were treated with ulixertinib and three patients (18%) had a PR, six had SD and eight had disease progression[166]. Although these results are encouraging for *NRAS*-mutant melanomas, they suggest that ulixertinib may not work for *KRAS*-mutant tumours. Because *NRAS*-mutant tumours have historically responded better to MEK and pan-RAF inhibitors than *KRAS*-mutant tumours, *KRAS*-mutant tumours should continue to be evaluated. Ulixertinib is currently being evaluated in combination with chemotherapy, nanoparticle albumin-bound paclitaxel (nab-paclitaxel) and gemcitabine in patients with metastatic PDAC in a phase I clinical trial (NCT02608229; TABLE 2).

KO-947 (FIG. 1), currently in a phase I clinical trial for *RAS*-mutant and *BRAF*-mutant NSCLC, potently reduced levels of phosphorylated ERK in preclinical models[167] (NCT03051035; TABLE 1). Sustained responses were observed; levels of phosphorylated ERK were suppressed for up to 5 days following a single dose in vitro[167]. These properties differ from those of the other ERK inhibitors discussed and suggest that KO-947 may provide a therapeutic advantage. However, this sustained suppression of phosphorylated ERK may not be well tolerated in patients.

LY-3214996 (FIG. 1), currently in a phase I clinical trial, is a potent and selective inhibitor in vitro (IC_{50} = 5 nM) of both ERK1 and ERK2 (REF.[168]) (NCT02857270; TABLE 1). In a dose-escalation arm, 33 patients with *RAS*-mutant and 16 with *BRAF*-mutant tumours were treated with LY-3214996 (REF.[169]). Seven patients with *BRAF* mutations had tumour regression, two had SD while one patient with *RAS*-mutant tumour had SD; the others had disease progression[169].

Overall, single-agent MEK, RAF or ERK inhibitors show little efficacy in the treatment of *RAS*-mutant tumours. Therefore, these inhibitors will have to be used in combination with other inhibitors of the MAPK pathway or with other approaches discussed in this Review. Finding the optimal combination for each type of inhibitor will be a challenge. However, the advent of allele-specific RAS inhibitors has increased the number of potential combinations that can be used to achieve maximal pathway suppression.

PI3K pathway inhibitors.—Of all the RAS effectors, the MAPK pathway has been the main focus for inhibition of *RAS*-mutant tumours. However, a second effector pathway, PI3K, is also activated by RAS. The next sections describe in more detail the types of PI3K inhibitors and the efficacy of these inhibitors for treating *RAS*-driven tumours.

There are three classes (I–III) of PI3Ks. Class I PI3Ks, upon activation by GTP-bound RAS, phosphorylate phosphatidylinositol 4,5-bisphosphate (PIP_2) to generate phosphatidylinositol (3,4,5)-trisphosphate (PIP_3), which recruits AKT to the membrane and allows activation of mTOR. Class I PI3Ks are generally composed of a heterodimer consisting of a catalytic subunit, p110, which contains an RBD, and a regulatory subunit, p85. Three genes (*PIK3CA*, *PIK3CB*, *PIK3CD* and *PIK3CG*) encode the four p110 subunit isoforms — p110α, p110β, p110γ and p110δ, respectively[170]. The p110α and p110β isoforms are ubiquitously expressed, whereas p110γ and p110δ are generally only expressed in immune cells[171,172]. The p110β and p110γ isoforms can be activated by both G protein-coupled receptors and receptor tyrosine kinases[173,174]. Cancers upregulate the PI3K pathway through activating mutations in *PIK3CA*, amplification of *AKT*, or loss of *PTEN*, which encodes a terminal phosphatase that converts PIP_3 to PIP_2 (REFS[175–177]). Interestingly, PI3K pathway mutations can coexist with *RAS* mutations, whereas *RAS* mutations and MAPK pathway mutations are mutually exclusive, such as *EGFR* or *BRAF*[4]. This suggests that *RAS* mutations are sufficient to dysregulate the MAPK but not the PI3K pathway. Activation of the PI3K pathway, which can occur in response to chemotherapy or MAPK inhibition, confers resistance to these two therapies[178]. Additionally, this suggests that the combined inhibition of MAPK and PI3K could be efficacious in treating RAS-driven tumorigenesis.

For the purpose of this Review, we discuss p110α inhibitors, as p110α is ubiquitously expressed and exclusively activated by RAS, unlike p110γ and p110δ. Four class I PI3K inhibitors have received FDA approval, and one of these, alpelisib, is p110α-specific, and one, copanlisib, is pan-class I PI3K (FIG. 1). These inhibitors are not approved for the treatment of *RAS*-mutant tumours.

RAS activates both the PI3K and MAPK pathways, and there are overlapping feedback mechanisms that provide crosstalk. Inhibition of one pathway can lead to the compensatory activation of the other; therefore, the inhibition of both MAPK and PI3K is a compelling strategy[179,180]. Preclinical models suggested that the combined inhibition of PI3K and MEK is efficacious and clinically achievable for the treatment of *RAS*-mutant tumours[181,182]. However, in clinical trials, the combination of these inhibitors was not tolerated and had little efficacy, presumably as a result of dose reduction due to toxicity[183–186].

To overcome this toxicity, studies focused on identifying receptor tyrosine kinases that could suppress PI3K, MAPK or both pathways. One such receptor, insulin-like growth factor 1 receptor (IGF1R), is required for mutant-RAS-mediated activation of PI3K[187,188]. Combined inhibition of IGF1R and MEK synergized effectively in CRC and NSCLC models[187,188]. Furthermore, replacing the MEK inhibitor with a KRAS-G12C covalent inhibitor in the combination with an IGF1R inhibitor, linsitinib, improved the efficacy and tolerability of this combination in mouse models[189]. The efficacy and tolerability of combinations of IGF1R and KRAS-G12C covalent inhibitors will need to be evaluated in a clinical setting.

No AKT inhibitors are currently approved for clinical use, in *RAS*-mutant tumours or otherwise (FIG. 1). The combination of AKT and MEK inhibitors is under clinical evaluation but toxicity issues similar to those observed in the PI3K–MAPK inhibitor studies

have been reported[190,191]. The combination of mTOR inhibitors (everolimus or temsirolimus) with MEK inhibitors (trametinib or pimasertib) also had poor tolerability[192,193] (FIG. 1). A recent review on targeting the PI3K pathway details the clinical status of AKT and mTOR inhibitors[194].

As the tissue type dictates the expression of p110 isoforms and the prevalence of *RAS* or *PI3K* mutations, further studies are required to determine the best setting for combinatorial approaches. Isoform-specific p110 inhibitors are expected to have fewer off-target effects and better toxicity profiles as they should more specifically target malignant cells. As p110δ/p110γ inhibitors have been approved for the treatment of chronic lymphocytic leukaemia and small lymphocytic lymphoma, and these isoforms are exclusively expressed in leukocytes, the use of these inhibitors could be efficacious in treating *RAS*-driven leukaemias. PI3K therapies should also be evaluated in combination with other approaches discussed in this Review, such as allele-specific inhibitors, including those for KRAS-G12C.

Emerging therapeutics

Small interfering RNA therapies

Systemic delivery of nanoparticles containing KRAS-targeting small interfering RNA (siRNA) in mouse models suggests that these could be effective approaches for targeting KRAS and could be mutant-specific[195]. However, a chemically modified antisense oligonucleotide, AZD4785, which significantly reduced levels of KRAS following subcutaneous injection in preclinical models, failed to sufficiently reduce KRAS levels in patients in a clinical trial (NCT03101839). Work is ongoing to improve uptake and internalization and to improve our understanding of how to make this approach more effective. A mutant-specific siRNA against KRAS-G12D, siG12D-LODER, showed promise in a phase I trial in combination with chemotherapy in 12 patients with PDAC; two patients achieved a PR while ten achieved SD (NCT01188785)[196]. An ongoing phase II trial in patients with $KRAS^{G12D}$ PDAC will evaluate siG12D-LODER in combination with gemcitabine and nab-paclitaxel (NCT01676259; TABLE 2).

Autophagy

Genetic suppression of *KRAS* with siRNA or short hairpin RNA or by pharmacological inhibition of the MAPK pathway using inhibitors against KRAS-G12C (ARS-853, ARS-1620), MEK (trametinib, cobimetinib) or ERK (SCH772984) increases autophagy[197,198]. PDAC cells have increased levels of autophagy and these cells require autophagy for growth, as pharmacological inhibition or genetic depletion of autophagy have induced tumour regression in preclinical models[199,200]. From these studies, a clinical study that inhibited autophagy with hydroxychloroquine, which is FDA-approved for the treatment of malaria, as a monotherapy was performed in 20 patients with PDAC and showed limited activity, as 18 of the 20 patients had disease progression[201,202]. Results from the use of hydroxychloroquine for preoperative care in combination with chemotherapy (gemcitabine plus nab-paclitaxel) were promising, as this treatment increased overall survival and decreased levels of the blood-borne tumour biomarker carbohydrate antigen 19–9 (CA 19–9)[203].

Although the treatment of PDACs with hydroxychloroquine had limited efficacy, the combination of hydroxychloroquine and inhibitors of the MAPK pathway showed promising results in preclinical models of PDAC and *NRAS*-mutant melanoma[197,198]. Combined treatment of hydroxychloroquine and trametinib resulted in robust tumour regression in xenograft and PDX models[197]. Treatment of a single patient with metastatic PDAC, who was refractory to all standard-of-care therapeutic options, with a combination of trametinib and hydroxychloroquine profoundly reduced CA 19–9 levels and induced a PR with a 50% reduction in tumour voume[197]. With this promising result, a phase I clinical trial to investigate the efficacy of trametinib plus hydroxychloroquine for the treatment of patients with PDAC is currently under evaluation (NCT03825289; TABLE 2).

Immunotherapy

Immune checkpoint inhibitors.—Tumours evade detection of the immune system through negative regulatory antigens (checkpoints). The widely discussed immune checkpoints include cytotoxic T lymphocyte protein 4 (CTLA4), PD1 and PDL1. CTLA4 negatively regulates T cell activation. PD1 is expressed on T cells and generates an intracellular inhibitory signal when bound to PDL1 (REF.[204]). Tumours express PDL1 on the cell surface, so this interaction inhibits immune activity in the vicinity of the tumour.

Seven antibodies that target immune checkpoint proteins have received FDA approval: one anti-CTLA4 (ipilimumab), three anti-PD1 (nivolumab, pembrolizumab, cemiplimab) and three anti-PDL1 (atezolizumab, avelumab, durvalumab) antibodies. Immunotherapies are approved for the treatment of NSCLC and melanoma, two of the four main *RAS*-mutant tumour types. Ipilimumab is currently approved as a monotherapy in metastatic melanoma, as an adjuvant therapy in melanoma[205,206] and in combination with nivolumab for the treatment of advanced NSCLC and melanoma[207,208]. The anti-PD1 antibodies nivolumab and pembrolizumab are currently approved for the treatment of unresectable or metastatic melanoma, as adjuvant therapy for melanoma (nivolumab) and for the treatment of advanced NSCLC[209–216]. The anti-PDL1 antibodies atezolizumab and durvalumab are also approved for the treatment of NSCLC (NCT02951767, NCT02108652)[217–219]. Clinical trials are underway to evaluate the efficacy of these anti-PDL1 antibodies for the treatment of melanoma (NCT02535078, NCT01772004)[220,221].

High mutational burden, high expression levels of PDL1 and increased numbers of tumour-infiltrating lymphocytes (TILs) are predictive of a response to immunotherapy[222–224]. PDAC and CRCs have low immunogenicity because they have immunosuppressive microenvironments and low mutational burdens[204,225–227]. Unsurprisingly, immunotherapies are not therapeutically beneficial in these tumours[228–230]. However, a subset of patients with PDAC or CRC that show high levels of microsatellite instability (MSI-H) or deficient mismatch repair (dMMR) respond to immunotherapies; fewer than 15% of CRCs and 20% of PDACs are MSI-H[231–234]. Given this success, pembrolizumab is approved for the treatment of MSI-H solid tumours and nivolumab is approved for MSI-H or dMMR CRC.

The clinical response to anti-PD1 and anti-PDL1 antibodies in NSCLC is limited to a subset of patients; roughly 10–20% of patients have a clinical response to these antibodies when used as single agents[235]. The mutational landscape of NSCLC can dictate the response to

checkpoint antibodies. In *KRAS*-mutant NSCLC, the presence of *LKB1* mutations reduces the overall response rate (7.4%) to PD1 blockade, whereas *TP53* mutations increase the response rate (35.7%) relative to tumours with *KRAS* mutations alone (28.6%)[236].

Combination treatment of allele-specific RAS inhibitors or inhibitors of the MAPK pathway and immunotherapies could improve the response of *RAS*-mutant tumours to immunotherapy. Interestingly, a clinical trial showed that NSCLC tumours with *KRAS* mutations had increased levels of PDL1, which could provide these tumours with immunoresistance[215]. Mutant KRAS directly upregulates PDL1 expression; this upregulation is reversed with a MEK inhibitor (trametinib) or a selective KRAS-G12C inhibitor (ARS-853)[237]. Additionally, in immune-competent mouse models, treatment with AMG 510 induced a pro-inflammatory tumour microenvironment, with an increased number of TILs, and the drug synergized with anti-PD1 treatment[32]. The combination of AMG 510 and anti-PD1 or anti-PDL1 for the treatment of NSCLC is being investigated in a phase II clinical trial (NCT03600883; TABLE 2). MEK inhibition also increases the number of TILs and, when combined with anti-PDL1 treatment, synergistically induces tumour regression[238]. Indeed, there is a phase II clinical trial investigating the efficacy of the combination of atezolizumab and cobimetinib in the treatment of NSCLC (NCT03600701; TABLE 2), although this combination did not provide a clinical benefit in metastatic CRC[145]. Additionally, a phase I clinical trial is currently evaluating spartalizumab, an anti-PD1 antibody, in combination with TNO155, a SHP2 inhibitor (NCT04000529; TABLE 2).

Adoptive cell therapy.—A second immunotherapeutic approach to treat RAS-driven cancers involves engineering the immune system to recognize antigens specific to mutant RAS proteins. This approach uses adoptive cell therapy to transfer T cells (either TILs or transgenic T cells), which have been expanded ex vivo, into patients. TILs recognize specific antigens displayed on tumours and transferring expanded TILs to patients provides clinical benefit in melanoma[239,240]. Technical developments in engineering T cell receptors (TCRs) and expressing them in peripheral blood lymphocytes achieves similar clinical benefits and allows the expansion of this technology beyond individual patients[241].

Given the successful identification of tumour-specific antigens in melanoma, a similar approach was used to identify *KRAS*-mutant specific antigens. From a patient with metastatic CRC, $CD8^+$ TILs were identified that specifically recognized KRAS-G12D and, upon infusion of the expanded TILs, the patient had tumour regression in seven pulmonary metastatic lesions with a durable PR lasting 9 months[242]. Another study identified KRAS-G12V-mutant-specific TCRs in $CD4^+$ T cells from a patient with NSCLC[243]. These studies showed that antigens from KRAS-G12D or KRAS-G12V are immunogenic in humans.

These antigens can then be used to engineer T cells. Wang and colleagues immunized mice bearing a transgene for a human leukocyte antigen, HLA-A*11:01, with mutant RAS peptides to generate T cells that recognized these mutations[244]. TCRs from the mouse T cells were identified, cloned and retrovirally transduced into peripheral blood lymphocytes to prime the T cells against RAS-G12D or RAS-G12V antigens[244]. In a xenograft model, these engineered mouse T cells detected human *KRAS*-mutant PDAC cells, leading to tumour reduction or complete regression[244]. Two clinical trials are currently using this

technology to transduce human peripheral blood lymphocytes with the murine TCRs against RAS-G12D or RAS-G12V in patients with HLA-A*11:01 (NCT03745326, NCT03190941; TABLE 1).

Cancer vaccines.—A third immunotherapeutic approach to treat *RAS*-mutant tumours uses known *RAS*-mutant tumour antigens to elicit T cell responses through vaccination. One such approach involves intradermal injection of peptides from mutant RAS proteins in combination with granulocyte–macrophage colony-stimulating factor (GM–CSF)[245]. This stimulation activates dendritic cells and triggers a T cell response against the mutant pepetides[245]. In a phase I/II clinical trial in patients with PDAC treated with a mutant-RAS-specific vaccine, Targovax TG-01, patients had an increased immune response and increased overall survival (NCT02261714)[246]. A second-generation vaccine, TG-02, has been used to treat patients with CRC but results have not been published (NCT02933944).

A second approach uses an mRNA that encodes neo-epitopes for common *KRAS* mutations (G12C, G12D, G12V and G13D)[247]. A lipid nanoparticle-formulated mRNA vaccine is given intramuscularly and the mRNA nanoparticle is taken up by antigen-presenting cells and translated and presented on the cell surface, which leads to T cell responses to the mutant RAS neo-epitopes. A phase I clinical trial is underway to evaluate one such mRNA vaccine, mRNA-5671, in patients with *KRAS* mutations, in which the mRNA is used either as a single agent or in combination with pembrolizumab (NCT03948763; TABLES 1,2).

Future directions and conclusions

KRAS-G12C allele-specific inhibitors will change the treatment landscape for RAS-driven tumours. These inhibitors are expected to be the first FDA-approved therapeutics for *RAS*-mutant tumours and will be used to treat refractory cancers driven by mutant RAS, such as PDAC, CRC and LUAD. Although the development of these inhibitors is incredibly exciting, new challenges and questions will arise.

There will be continued development of inhibitors specific to other alleles, such as KRAS-G12D and KRAS-G12V. These alleles are the most common *KRAS* variants and are therefore associated with the largest patient populations. Eventually, specific inhibitors could be developed for all mutant RAS alleles, providing a personalized medicine approach. Targeting mutant RAS proteins is the best approach for *RAS*-mutant tumours; however, allele-specific inhibitors will probably have limited efficacy as monotherapies. The greatest antitumour effects will require combinations with other inhibitors.

Determining which combination strategies will work best in patients will be challenging for several reasons. First, each variation in RAS has distinct biochemical properties and these properties will determine the response to many of the therapeutic approaches discussed. For example, SHP2 inhibition using RMC-4550 was effective in treating cells with $KRAS^{G12}$ mutations, and was more effective against $KRAS^{G12C}$ than against $KRAS^{G12D}$ or $KRAS^{G12V}$ (REF.[63]). This observation suggests that a combination of SHP2 and KRAS-G12C inhibitors would be an effective therapeutic strategy. Understanding both the

requirements of specific mutant RAS codons and the response of inhibitors to these alleles will be necessary in developing strategic combination therapies.

Second, as discussed throughout this Review, the tumour type can dramatically impact the response rate. *RAS*-mutant CRC and PDAC have a minimal response to inhibitors of MAPK or immune checkpoint blockade. Early data from the AMG 510 trial showed that CRC is more refractory to treatment than LUAD, suggesting that CRC will require combination therapies[1]. CRC tumours are particularly challenging to treat. However, promising antitumour effects have been observed in $BRAF^{V600E}$ metastatic CRCs treated with the triple combination of binimetinib, encorafenib and cetuximab, suggesting that *KRAS*-mutant CRC will require an aggressive combination strategy to achieve a response[248].

Third, historically, combination treatments have been toxic and have poor safety profiles. However, the lack of dose-limiting toxicities observed with AMG 510 is encouraging and mutant-specific therapies should have limited off-target effects. Allele-specific inhibitors (with low toxicity) could be combined with other inhibitors that have greater toxicity, without encountering the problems observed with combining two toxic compounds, as occurred with combinations of the PI3K and MEK inhibitors.

As the treatment of RAS-driven tumours becomes more personalized, potential resistance mechanisms to allele-specific inhibition need to be evaluated. The heterogeneity of tumours could provide intrinsic mechanisms of resistance. For example, one tumour may contain 95% $KRAS^{G12C}$ and 0.1% $KRAS^{G12V}$ cells. Upon treatment with an allele-specific KRAS-G12C inhibitor, the tumour will regress but ultimately the $KRAS^{G12V}$ cells will be selected for and the tumour will relapse. Tumour heterogeneity also can lead to intrinsic resistance, as subpopulations of cells are resistant to KRAS-G12C inhibition[249]. Preclinical models have not accounted for tumour heterogeneity and responses in the clinic will clarify this effect. Currently, there is little evidence regarding the types of de novo mutations that will arise upon treatment with KRAS-G12C allele-specific inhibitors.

The mechanisms of resistance that have been described for EGFR and BRAF-V600 inhibitors may shed some light as to what de novo mutations we might expect to see clinically with allele-specific KRAS-G12C inhibitors. Cysteine mutations are one expected mechanism of resistance, as these have been observed for covalent EGFR inhibitors[250,251]; however, in the case of KRAS-G12C, the cysteine mutation is required for oncogenic activity and hence it is less likely that this will be observed clinically unless the residue is mutated to another oncogenic KRAS-G12-mutant allele (such as to an aspartate, valine or other residue). Gene amplification, including *BRAF* or *RAS*, has been described as a mechanism of resistance to BRAF-V600 inhibitors[252–254], and *MET* amplification has been observed in the context of EGFR inhibitors[255,256]. In this case, amplification of *EGFR* or another upstream EGFR family member could drive elevated GTP-bound RAS levels in cells, conferring resistance to the KRAS-G12C inhibitors as these molecules bind to the GDP-bound form. Additionally, *RAS* amplification, either *HRAS* or *NRAS*, or amplification of *KRAS* itself could potentially drive RAS dimerization and elevated GTP-bound RAS levels, thereby conferring resistance to the KRAS-G12C inhibitors. The majority of acquired resistance mechanisms in *BRAF*-mutant tumours reactivate the MAPK pathway and, in

addition to the above mechanisms, this can be achieved by mutations in *NRAS* and *MEK1/2* (REFS[257–259]). Therefore, reactivation of the MAPK pathway poses numerous conceivable mechanisms of resistance to allele-specific inhibitors and could be achieved through either altering upstream signalling (through *EGFR* mutations or amplification or *RAS* amplification), or downstream mechanisms, such as *RAF* or *MEK* mutations. Reactivation of *EGFR* can also occur through autocrine and/or paracrine mechanisms such as secretion of proheparin-binding EGF-like growth factor[249].

Cell state changes, such as a switch from NSCLC to SCLC[260,261] or upregulation of the master regulator MITF[262,263], in melanoma have been described as resistance mechanisms to EGFR and BRAF-V600 inhibitors. Additionally, epithelial–mesenchymal transition reprogramming has been associated with resistance to EGFR inhibition in NSCLC[249,260,264,265] and with BRAF-V600 inhibition in melanoma[266,267]. Certainly, transcriptional mechanisms of resistance to KRAS-G12C inhibitors could ultimately render the tumour cell state independent of mutant KRAS. Each of these resistance mechanisms will likely require novel combination approaches in order to achieve more complete and durable suppression of RAS-driven tumours.

Mutations that disable the GTPase activity of RAS are another hypothetical mechanism of resistance. In agreement with this hypothesis, a CRISPR screen showed that loss of *NF1* promotes resistance[37]. Importantly, loss of *NF1* renders $BRAF^{V600E}$ tumours resistant to vemurafenib treatment, suggesting that *RAS*-mutant tumours may utilize a similar approach[268]. Additionally, when a mutation blocking GTPase activity (A59G) was introduced in conjunction with KRAS-G12C, allele-specific KRAS-G12C inhibitors had a diminished effect as KRAS-G12C was predominately in the GTP-bound state[34,35]. However, it is unclear whether these predicted mechanisms would be consistent across *RAS* mutations or tumour types.

This is an exciting time to be at the forefront of ‘drugging the undruggable’ as there is new optimism that *RAS*-mutant cancers can be successfully treated.

Glossary

Allosteric	A site that is outside the active site of an enzyme.
RASopathies	A group of clinically defined genetic syndromes caused by germline mutations of regulators or components of the MAPK pathway.
Noonan syndrome	An autosomal dominant RASopathy characterized by distinctive craniofacial features. Frequently germline mutated genes in Noonan syndrome include *PTPN11*, *SOS1*, *RAF1*, *KRAS*, *NRAS*, *MRAS*, *SHOC2*, *CBL* and *RIT1*.

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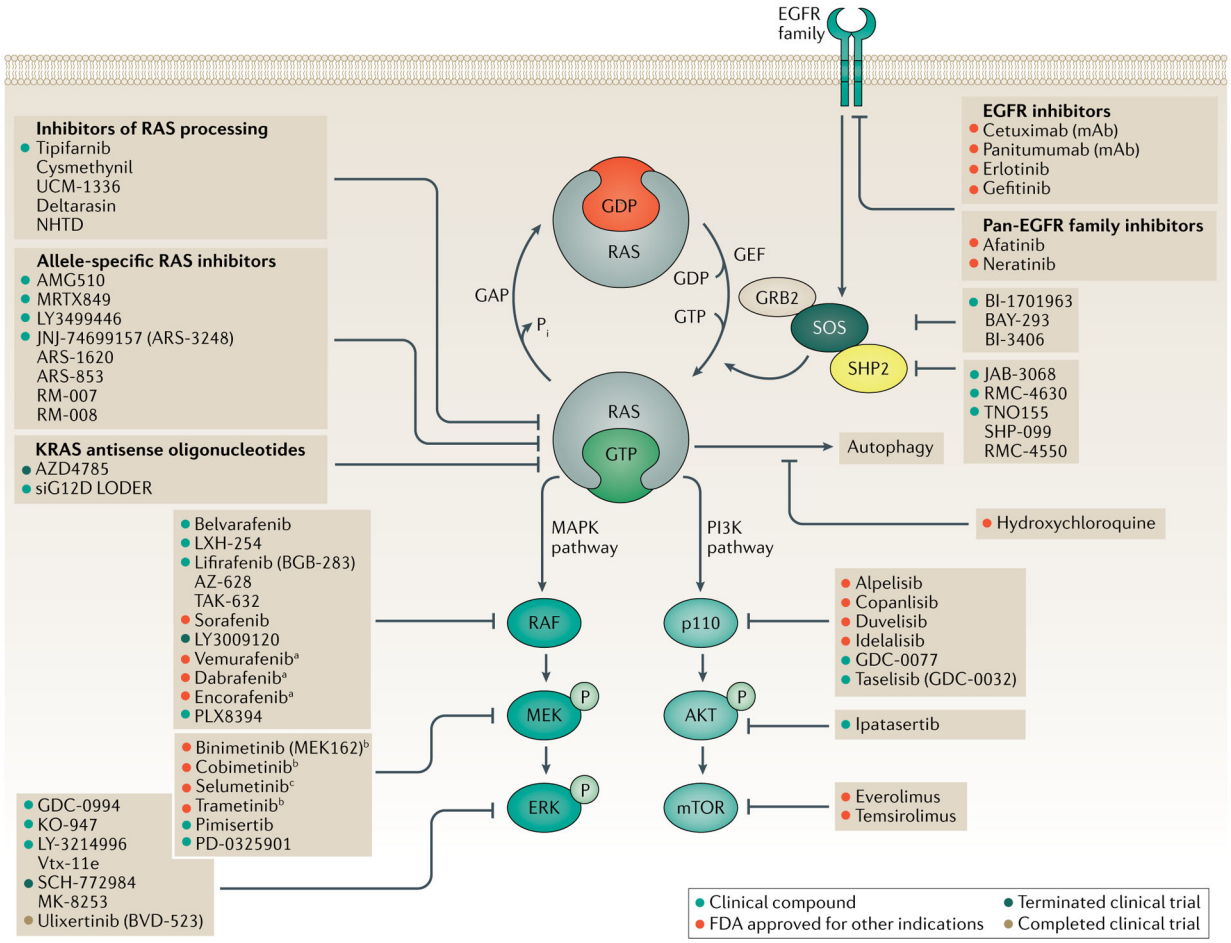

Fig. 1 |. Clinical development of inhibitors for *RAS*-mutant tumours.

Activation of receptor tyrosine kinases, such as members of the epidermal growth factor receptor (EGFR) family, promotes the exchange of GDP for GTP in RAS, thereby activating RAS. Inhibition of EGFR can reduce this activation. Inhibition of SOS or SHP2 decreases the rate of GDP–GTP exchange and reduces the GTP-bound RAS population. Mutant RAS proteins accumulate in the GTP-bound state. A number of approaches have been developed to directly inhibit RAS, including covalent allele-specific inhibitors that bind to KRAS-G12C. GTP-bound RAS activates downstream signalling by binding to the RAS-binding domain of effector proteins, such as RAF and p110, to activate the MAPK and PI3K signalling cascades, respectively. Both the MAPK and PI3K signalling cascades can be inhibited at each kinase tier. Data compiled from ClinicalTrials.gov and AccessData.FDA.gov. [a]Only effective against monomeric BRAF (BRAF-V600E/K). [b]Approved for the treatment of *BRAF*-mutant melanoma. [c]Approved for the treatment of paediatric patients with *NF1* mutations.

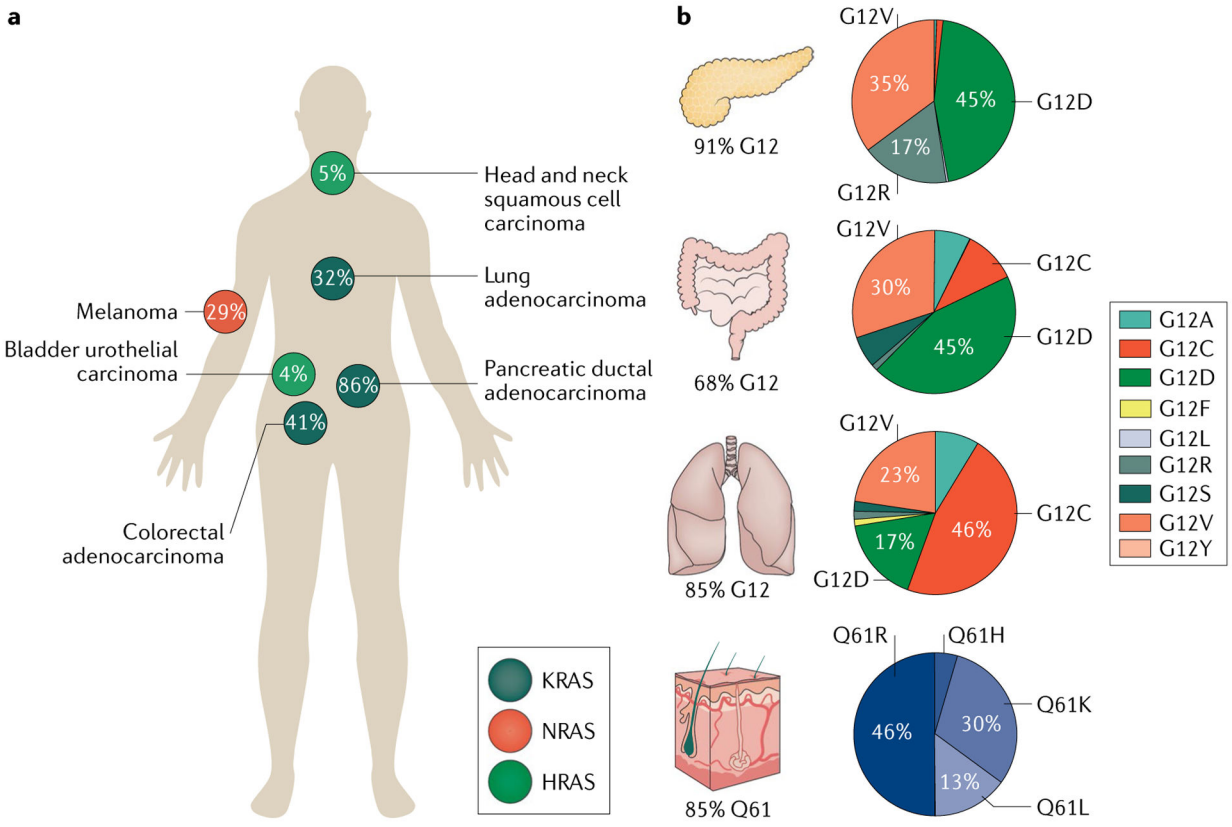

Fig. 2 |. Frequency and distribution of RAS mutations in human cancers.

Human cancers differ in which has the most frequently mutated *RAS* isoform, codon and amino acid substitution. **a** | Distribution of *RAS* isoform (*KRAS*, *NRAS* and *HRAS*) mutations across tumour types and the frequency of the *RAS* mutation by isoform in each tumour type. **b** | Percentages of *KRAS* mutations that are in codon 12 by tissue type for pancreatic, colorectal and lung adenocarcinoma, and the percentage of *NRAS* mutations that are in codon 61 for melanoma. The distributions of amino acid substitutions at the mutated codon (12 or 61) for each tissue type are shown in pie charts beside the relevant organ. Data acquired from The Cancer Genome Atlas (pan-Cancer) from cBioPortal and from Project GENIE[269] (GENIE v7.0 public).

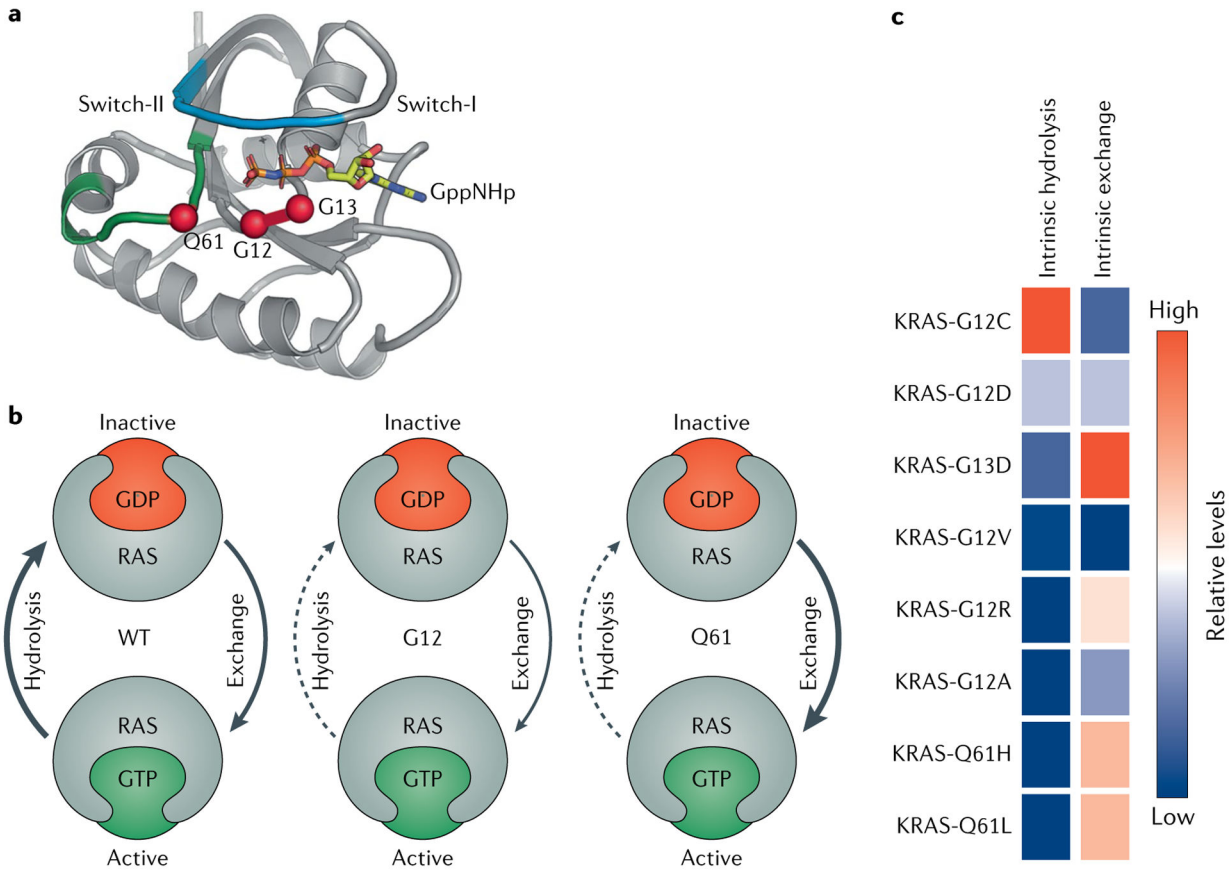

Fig. 3 |. Biochemical features of mutant RAS proteins.

Mutations in codons 12, 13 and 61 disrupt the GTP hydrolysis and guanine exchange rates of RAS proteins. **a** | Ribbon diagram of HRAS. Switch-I is shown in light blue, switch-II is in green, GppNHp (a GTP analogue) is shown in yellow, and oncogenic hotspot residues G12, G13 and Q61 are shown as red spheres. **b** | Summary of generalized biochemical disruption of hydrolysis and guanine exchange upon mutations in codon 12 or 61. Generally, mutations in codon 12 disrupt the GTPase activity of RAS and thereby decrease the rate of GTP hydrolysis, so the mutant protein accumulates in the GTP-bound state. Mutations in codon 61 accelerate the rate of GDP–GTP exchange and simultaneously decrease the rate of GTP hydrolysis, so codon 61 RAS mutants also accumulate in the GTP-bound state. **c** | Biochemical properties of specific amino acid substitutions at KRAS codon 12, 13 or 61, rank ordered by intrinsic hydrolysis. Data acquired from results in Hunter et al.[23]. WT, wild type.

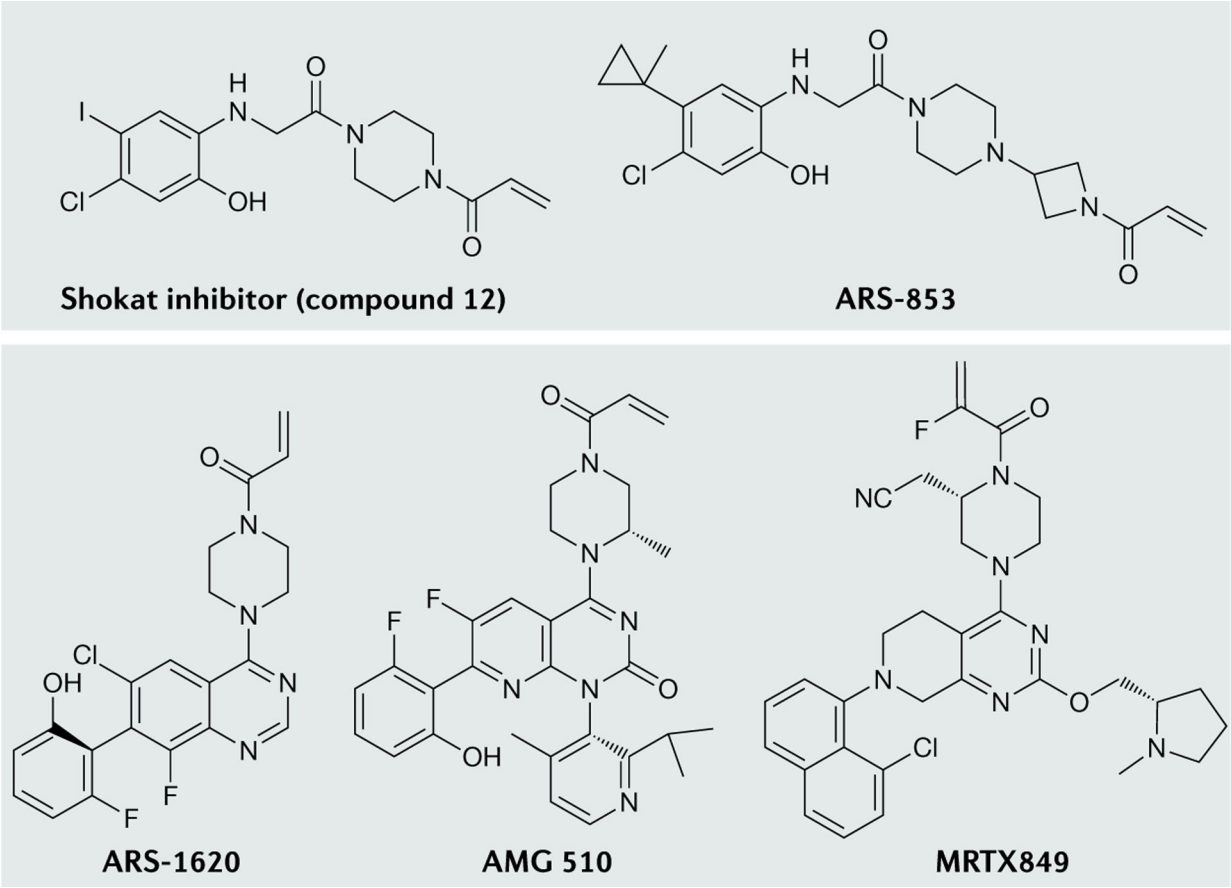

Fig. 4 |. Chemical structures of compounds that bind to KRAS-G12C.
Shokat and colleagues developed the first series of small molecules to bind KRAS-G12C, the most potent of which is compound 12 (REF.[24]). Modification of the linker and hydrophobic binding pocket led to the development of a more potent and cellular active compound, ARS-853 (REF.[36]). Further improvements, such as the introduction of a quinazoline-based series and a fluorophenol hydrophobic binding moiety, enhanced the potency and pharmacological properties and led to the development of ARS-1620 (REF.[30]). Using an alternative orientation of His95 in the switch-II pocket allowed the addition of aromatic rings to enhance the protein–protein interactions with KRAS-G12C, leading to the development of AMG 510 (REF.[32]). Structure-based drug design approaches and optimization led to the development of MRTX849 (REF.[34]).

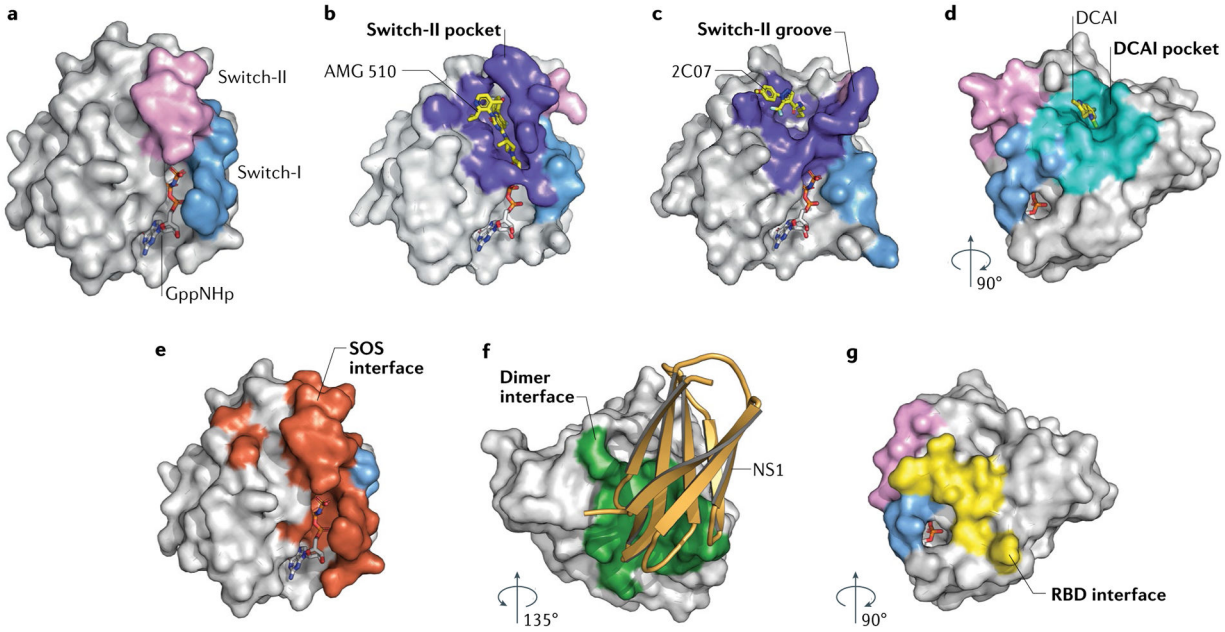

Fig. 5 |. Structures of RAS surfaces targeted by therapeutics.

Proteins are depicted by surface representation, and compounds and nucleotides are shown as stick models. Each panel is coloured to highlight important surfaces, with switch-I in blue, switch-II in pink and relevant interfaces coloured uniquely. **a** | HRAS binds to GppNHp, a non-hydrolysable nucleotide, which can be used as a reference for orientation (Protein Data Bank (PDB): 5P21). **b** | Switch-II pocket (purple) of KRAS-G12C bound to AMG 510 (PDB: 6OIM; compound identifier: MOV). **c** | Switch-II groove (purple) bound to 2C07 (PDB: 5VBZ; compound identifier: 92V). **d** | DCAI pocket (teal) of KRAS-G12D bound to the DCAI compound (PDB: 4DST; compound identifier: 9LI). **e** | Son of Sevenless (SOS) binding interface (red) of HRAS (PDB: 1BKD). **f** | Proposed HRAS dimerization interface (green) bound by NS1 monobody (gold) (PDB: 5E95). **g** | RAF–RAS-binding domain (RBD) binding interface (yellow) of HRAS (PDB: 4G0N).

Table 1 |

Single-agent inhibitors in clinical development

Drug	Biomarker	Disease setting	Study phase	ClinicalTrials.gov registration
KRAS-G12C inhibitors				
AMG 510	*KRAS*^{G12C} mutation	Advanced solid tumours	I/II	NCT03600883
MRTX849	*KRAS*^{G12C} mutation	Advanced solid tumours	I/II	NCT03785249
JNJ-74699157 (ARS-3248)	*KRAS*^{G12C} mutation	Advanced solid tumours	I	NCT04006301
LY3499446	*KRAS*^{G12C} mutation	Advanced solid tumours	I/II	NCT04165031
SOS inhibitors				
BI-1701963	*KRAS* mutations	Advanced or metastatic solid tumours	I	NCT04111458
SHP2 inhibitors				
RMC-4630	Mutations that hyperactivate the MAPK pathway	Relapsed or refractory solid tumours	I	NCT03634982
TNO155	*EGFR* or *KRAS*^{G12C} mutations	Advanced solid tumours	I	NCT03114319
Farnesyltransferase inhibitors				
Tipifarnib	*HRAS* mutations	Thyroid cancer, squamous head and neck cancer and squamous cell carcinoma	II	NCT02383927
RAF inhibitors				
Belvarafenib (HM95573)	*BRAF, KRAS* or *NRAS* mutations	Advanced solid tumours	I	NCT03118817
LXH-254	MAPK pathway mutation	Advanced solid tumours	I	NCT02607813
MEK inhibitors				
Binimetinib (MEK162)	*NRAS* mutation	Unresectable or metastatic melanoma	III	NCT01763164
ERK inhibitors				
KO-947	*BRAF, KRAS, NRAS* or *HRAS* mutation	Advanced solid tumours	I	NCT03051035
LY-3214996	*BRAF* or *NRAS* mutations	Metastatic melanoma and NSCLC	I	NCT02857270
Adoptive cell therapies				
Anti-RAS-G12D mTCR	HLA-A11:01 *RAS*^{G12D} mutation	Advanced solid tumours	I/II	NCT03745326
Anti-RAS-G12V mTCR	HLA-A11:01 *RAS*^{G12V} mutation	Advanced solid tumours	I/II	NCT03190941
Cancer vaccines				
mRNA-5671	HLA-A11:01 and/or HLA-C08:02; *KRAS*^{G12C}, *KRAS*^{G12D}, *KRAS*^{G12V} or *KRAS*^{G13D} mutation	NSCLC, non-MSI-H CRC, PDAC	I	NCT03948763

CRC, colorectal cancer; HLA, human leukocyte antigen; MSI-H, microsatellite instability-high; mTCR, murine T cell receptor; NSCLC, non-small-cell lung cancer; PDAC, pancreatic ductal adenocarcinoma; SOS, Son of Sevenless.

Table 2 |

Combination therapies in clinical development

Drugs	Biomarker	Disease setting	Study phase	ClinicalTrials.gov registration
KRAS-G12C combinations				
AMG510 and antibodies to PD1 or PDL1	*KRAS*^{G12C} mutation	Advanced NSCLC	II	NCT03600883
MRTX849 and TNO155	*KRAS*^{G12C} mutation	Advanced or metastatic solid tumours	I/II	NCT04330664
LY3499446 and abemaciclib	*KRAS*^{G12C} mutation	Advanced solid tumours	I/II	NCT04165031
LY3499446 and cetuximab	*KRAS*^{G12C} mutation	Advanced solid tumours	I/II	NCT04165031
LY3499446 and erlotinib	*KRAS*^{G12C} mutation	Advanced solid tumours	I/II	NCT04165031
LY3499446 and docetaxel	*KRAS*^{G12C} mutation	Advanced solid tumours	I/II	NCT04165031
SOS *inhibitor combinations*				
BI-3406 and trametinib	*KRAS* mutation	Advanced or metastatic solid tumours	I	NCT04111458
SHP2 inhibitor combinations				
TNO155 and spartalizumab	*EGFR* or *ALK* WT NSCLC	Advanced solid tumours	Ib	NCT04000529
TNO155 and ribociclib	WT *EGFR* or WT *ALK* NSCLC, *KRAS*-mutant CRC or NSCLC	Advanced solid tumours	Ib	NCT04000529
RAF inhibitor combinations				
Belvarafenib and cobimetinib	*RAS* or *RAF* mutations	Locally advanced or metastatic tumours	Ib	NCT03284502
LXH-254 and an antibody to PD1	*NRAS*-mutant melanoma and *KRAS*-mutant NSCLC	Advanced solid tumours	I	NCT02607813
LXH-254 and trametinib	*KRAS-mutant* or *BRAF*-mutant NSCLC or *NRAS*-mutant melanoma	Advanced or metastatic solid tumours	Ib	NCT02974725
LXH-254 and LTT462	*KRAS*-mutant or *BRAF*-mutant NSCLC or *NRAS*-mutant melanoma	Advanced or metastatic solid tumours	Ib	NCT02974725
LXH-254 and ribociclib	*KRAS*-mutant or *BRAF*-mutant NSCLC or *NRAS*-mutant melanoma	Advanced or metastatic solid tumours	Ib	NCT02974725
BGB-283 and PD-0325901	*KRAS*-mutant NSCLC or endometrial cancer	Advanced or refractory solid tumours	Ib	NCT03905148
MEK inhibitor combinations				
Cobimetinib and atezolizumab	*KRAS* mutation	Advanced and metastatic NSCLC	II	NCT03600701
Cobimetinib and RMC-4630	Mutations that hyperactivate the MAPK pathway	Relapsed or refractory solid tumours	Ib/II	NCT03989115
Selumetinib and afatinib	*KRAS* mutation, WT *PIK3CA*	Advanced and metastatic NSCLC	I/II	NCT02450656
Selumetinib and MK-8353	*RAS* or *RAF* mutation	Advanced solid tumours	Ib	NCT03745989
Trametinib and ponatinib	*KRAS* mutation	Advanced NSCLC	I	NCT03704688

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Drugs	Biomarker	Disease setting	Study phase	ClinicalTrials.gov registration
Trametinib and hydroxychloroquine	None	Advanced pancreatic cancer	I	NCT03825289
ERK inhibitor combinations				
MK-8353 and pembrolizumab	None	Advanced solid tumours and CRC	Ib	NCT02972034
Ulixertinib (BVD-523) and nab-paclitaxel + gemcitabine	None	Metastatic pancreatic cancer	Ib	NCT02608229
Ulixertinib (BVD-523) and palbociclib	None	Advanced or metastatic pancreatic cancer or solid tumours	I	NCT03454035
LY-3214996 and midazolam	*BRAF* or *RAS* mutations	Advanced or metastatic solid tumours	I	NCT02857270
LY-3214996 and abemaciclib	*BRAF*or *RAS* mutations	Advanced or metastatic solid tumours	I	NCT02857270
LY-3214996 and nab-paclitaxel + gemcitabine	*KRAS* mutations	Advanced or metastatic solid tumours	I	NCT02857270
siRNA combinations				
siG12D-LODERand nab-paclitaxel + gemcitabine	$KRAS^{G12D}$ mutation	Unresectable locally advanced pancreatic cancer	II	NCT01676259
Cancer vaccine combinations				
mRNA-5671 and pembrolizumab	HLA-A11:01 and/or HLA-C08:02 $KRAS^{G12C}$, $KRAS^{G12D}$, $KRAS^{G12V}$ or $KRAS^{G13D}$ mutation	NSCLC, non-MSI-H CRC, PDAC	I	NCT03948763
Tyrosine kinase inhibitor combinations				
Neratinib and divalproex sodium	*KRAS* or *NRAS* mutation	Advanced solid tumours	I/II	NCT03919292
Cetuximab and irinotecan	RAS^{G131} mutation	Advanced colorectal cancer	II	ACTRN12612 000901808

CRC, colorectal cancer; HLA, human leukocyte antigen; MSI-H, microsatellite instability-high; nab-paclitaxel, nanoparticle albumin-bound paclitaxel; NSCLC, non-small-cell lung cancer; PDAC, pancreatic ductal adenocarcinoma; SOS, Son of Sevenless; TBA, to be advised; WT, wild type.