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Targeting KRAS G12C: from inhibitory mechanism to modulation of antitumor effect in patients

Dongsung Kim[1,+], **Jenny Xue**[1,2,+], **Piro Lito**[1,2,3,4,*]

[1]Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer, New York, NY

[2]Weill Cornell/Rockefeller/Sloan Kettering Tri-Institutional MD-PhD Program, New York, NY

[3]Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY

[4]Department of Medicine, Weill Cornell Medical College, New York, NY

Abstract

KRAS mutations are among the most common genetic alterations in lung, colorectal and pancreatic cancers. Direct inhibition of KRAS oncoproteins has been a longstanding pursuit in precision oncology, one established shortly after the discovery of RAS mutations in human cancer cells nearly 40 years ago. Recent advances in medicinal chemistry have established inhibitors targeting KRAS(G12C), a mutation found in ~13% of lung adenocarcinomas and, at a lower frequency, in other cancers. Preclinical studies describing their discovery and mechanism of action, coupled with early clinical data from patients treated with these drugs, have sparked a renewed enthusiasm in the study of KRAS and its therapeutic potential. Here we discuss how these advances are reshaping the fundamental aspects of KRAS oncoprotein biology and the strides being made towards improving patient outcomes in the clinic.

KRAS and the highly-related NRAS and HRAS GTPases hydrolyze guanosine triphosphate (GTP) to guanosine diphosphate (GDP). They control diverse cellar functions by cycling between an active, GTP-bound, and an inactive, GDP-bound, conformation (reviewed in Hobbs et al., 2016; Pylayeva-Gupta et al., 2011). The GTPase activity of KRAS is enhanced by GTPase-activating proteins (GAP), such as NF1, and the exchange of GDP for GTP is enhanced by guanine-nucleotide exchange factors (GEF), such as SOS1/2 (Bos et al., 2007; Margarit et al., 2003; Trahey and McCormick, 1987). The balance between hydrolysis and exchange determines the levels of active KRAS in cells.

*Correspondence: litop@mskcc.org.
+These authors contributed equally

Conflict of interests

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KRAS activates several effector proteins to control diverse cellular functions (reviewed in Downward, 2003; Malumbres and Barbacid, 2003). The best studied effectors include RAF kinases and the catalytic subunit of PI3K. KRAS-GTP binding to RAF kinases stimulates their dimerization and activation, which triggers the step-wise activation of MEK and ERK, a pathway that drives cell-cycle progression and proliferation. By binding to PI3K, KRAS helps activate AKT and mTOR, which regulate apoptosis, metabolism and translation.

Effective targeting of KRAS signaling has been difficult to achieve in patients (reviewed in Ostrem and Shokat, 2016; Papke and Der, 2017). Competitive inhibition of nucleotide binding, as that afforded for kinases, has not been feasible for RAS GTPases because of their high affinity for the nucleotide. Blocking the localization of KRAS at the plasma membrane, a key component for its activation, has been ineffective because multiple compensatory pathways regulate this process. Similarly, targeting effector signaling downstream of KRAS has not yielded significant clinical benefit (reviewed in Karoulia et al., 2017; Lito et al., 2013), either because of paradoxical signaling activation triggered by the inhibitor (e.g. RAF inhibitors) or because on-target toxicity limiting the maximum tolerated dose in patients (e.g. MEK or AKT inhibitors). Considering this historical context and the prevalence of KRAS activating mutations in cancer patients, the discovery and clinical development of KRAS(G12C) selective inhibitors is heralding a new era in precision oncology. Here we review the evolution of these exciting new therapies, their mechanism of action and cellular effects in preclinical studies, as well as the initial observations from patients treated with KRAS(G12C) inhibitors in clinical trials.

KRAS activation in cancer

KRAS mutations are found predominantly in lung (approximately 25% of cases and estimated to affect 57,000 patients per year in the US), pancreatic (~95% of cases and ~54,000 patients) and colorectal (~35% of cases and ~36,000 patients) cancers. Mutations in NRAS and HRAS are also found in cancer patients, but at a lower frequency than KRAS. KRAS mutations result in single amino acid substitutions that activate the oncoprotein by hindering its ability to hydrolyze GTP (reviewed in Simanshu et al., 2017). Substitutions at the G12 (e.g. D/C/V/R/A/S) or G13 (e.g. D/C/V) residues prevent the stabilization of the hydrolysis transition state by a critical arginine residue in GAPs (Bollag and McCormick, 1991; Scheffzek et al., 1997). Less common variants, such as Q61H/L/R/K, interfere with the coordination of the water molecule involved in hydrolysis (Scheidig et al., 1999), whereas A146T enhances the propensity for nucleotide-exchange (Poulin et al., 2019). The distribution of KRAS mutant alleles differs across tumors, with G12C comprising ~50% of KRAS mutations in lung cancer and G12D being the most common allele in pancreatic and colorectal cancers (Li et al., 2018a; Riely et al., 2008). In addition to being found in ~13% of patients with lung adenocarcinoma, the KRAS(G12C) allele is found at a lower frequency in those with colorectal (~3%), uterine (~2%), mesothelioma (~1%), pancreatic (<1%), cervical (<1%), bladder (<1%) or gastric (<1%) cancers (the frequency estimates are from the cancer genome atlas (TCGA) and www.cbioportal.org).

While insensitive to GAP-assisted hydrolysis, RAS oncoproteins have measurable intrinsic GTP hydrolysis rates in biochemical assays (Gibbs et al., 1988; Hunter et al., 2015; Smith et

al., 2013; Wey et al., 2013). This was believed to be inconsequential for cellular function and KRAS oncoproteins were traditionally viewed as being ‘constitutively’ active. Recent studies by us and others are reshaping this notion, by showing that some KRAS oncoproteins cycle between their active and inactive states in cancer cells and are dependent on nucleotide-exchange for activation (Lito et al., 2016; Nichols et al., 2018; Patricelli et al., 2016; Poulin et al., 2019; Rabara et al., 2019). As discussed below, this has important implications for their susceptibility to therapy.

Allele-specific inhibitors targeting a mutated cysteine residue

Direct KRAS oncoprotein inhibition has been a long-standing objective in precision medicine. The earliest efforts track back to the 1980’s, when RAS activating mutations were found in human cancer cells and not long after the discovery of RAS oncogenes in transforming viruses. The discovery of inhibitors that selectively target KRAS(G12C) (hereafter referred to as G12Ci), while sparing wild-type or other mutant KRAS (Ostrem et al., 2013), was a groundbreaking advance in this quest. The inhibitors bind covalently to the mutant cysteine residue, and occupy a pocket in the switch-II region (SIIP), when KRAS(G12C) is in its inactive, GDP-bound, state (Figure 1A). The drugs are expected to also bind to $NRAS^{G12C}$ and $HRAS^{G12C}$; although these variants are very rare: $NRAS^{G12C}$ is estimated to be present in ~0.5% of acute myeloid leukemias and colorectal cancers, and $HRAS^{G12C}$ in ~0.4% of head and neck cancers. As such, the effect of G12Ci on these oncoproteins has not been scrutinized experimentally.

Structure-based optimization led to inhibitors with progressively higher affinities for KRAS(G12C), including ARS853 (Lito et al., 2016; Patricelli et al., 2016), 1_AM (Zeng et al., 2017), ARS1620 (Janes et al., 2018). These suppress KRAS(G12C) activation, attenuate proliferation and induce cell-death to varying degrees across tumor models. Importantly, ARS1620 inhibits tumor growth in xenograft models (Janes et al., 2018), consolidating inactive state-selective inhibition as an effective approach for suppressing KRAS(G12C) signaling.

Being made publicly available from early on, the structure-activity relationships of the ARS compounds furthered the development of even more potent G12Ci. While similar in that they share an acrylamide warhead that engages G12C, these inhibitors have distinct chemical structures (Figure 1A). Two of these, sotorasib (also known as AMG510, Canon et al., 2019) and MRTX849 (Hallin et al., 2020), have antiproliferative IC_{50}s in the low nanomolar range and potently suppress xenograft tumor growth in mice. Their enhanced potency reportedly owes to an enhanced interaction with the H95 residue in the α3 helix of KRAS(G12C) (Fell et al., 2020; Lanman et al., 2020), an interaction that is also reported to be responsible for the improved potency of ARS1620 and 1_AM over ARS853 (Janes et al., 2018; Zeng et al., 2017). Excitingly, sotorasib and MRTX849 now show clinical activity in patients with KRAS(G12C)-mutant tumors.

Other evolving approaches to target KRAS(G12C) include GDP-analogues (Hunter et al., 2014), compounds that displace effector-binding (Kessler et al., 2019) and ‘tri-complex’, KRAS(G12C)-GTP:cyclophylin A (CYPA) inhibitors (Nichols et al., 2020). The cellular

effects of these emerging compounds are under evaluation and it remains to be seen if they achieve the same or better KRAS inhibition than SIIP inhibitors.

Mechanism of inactive state-selective KRAS(G12C) inhibition

How do inactive state-selective drugs inhibit KRAS(G12C), an oncoprotein conventionally thought to be constitutively active? Key to answering this question is the realization that KRAS(G12C) undergoes nucleotide cycling in cancer cells and fluctuates between its active and inactive states (Lito et al., 2016). When introduced alongside KRAS(G12C), secondary mutations that completely block hydrolysis, such as A59G or Q61L, increase baseline KRAS(G12C) activation and attenuate the response to G12Ci-treatment. Experiments using mass spectrometry to quantify drug-bound KRAS(G12C) following perturbations of its nucleotide cycle in cells and studies utilizing more potent inhibitors, reached a similar conclusion (Hallin et al., 2020; Patricelli et al., 2016). Thus, an intact GTPase activity is required for KRAS(G12C) inhibition.

Drug-binding to KRAS(G12C) prevents its reactivation by nucleotide-exchange and traps the oncoprotein in the inactive state (Figure 1C). This is supported by experiments in which drug-bound KRAS(G12C) cannot undergo SOS1- or EDTA- mediated nucleotide-exchange (Canon et al., 2019; Janes et al., 2018; Lito et al., 2016; Patricelli et al., 2016). Initially, drug-binding was thought to lower the affinity of KRAS(G12C) for GTP, however, the drug also attenuates the exchange of GDP for GDP (Janes et al., 2018; Patricelli et al., 2016), suggesting a primary effect on nucleotide-exchange rather than affinity. Indeed, the potency of G12Ci-treatment is attenuated when secondary mutations enhancing nucleotide-exchange are engineered alongside KRAS(G12C) (Lito et al., 2016). In a similar manner, inhibition of SOS1 enhances KRAS(G12C) inhibition (Hillig et al., 2019).

A question yet to be answered is how does KRAS(G12C) undergo sufficient hydrolysis to enable inhibition by inactive state-selective drugs? It has been proposed that KRAS(G12C) is unique among commonly occurring KRAS mutants, in that it has a high intrinsic hydrolysis rate (Hunter et al., 2015; Simanshu et al., 2017). Whether this is sufficient to satisfy the kinetics of drug trapping in cells, however, remains unknown.

Initial clinical effects

Indicative of the tremendous progress made in the seven years since their discovery, several inactive state-selective KRAS(G12C) inhibitors are now in clinical trials (Table I). These include sotorasib, MRTX849, GDC6036, LY3499446 and JNJ74699157 (ARS3248). Others are anticipated to enter the clinic in the coming year.

The clinical data so far suggest a wide-therapeutic index for some G12Ci, namely sotorasib and MRTX849. Treatment with these drugs appears well-tolerated by most patients, although side effects including diarrhea, anemia or liver enzyme abnormalities have been reported (Christensen et al., 2019; Govindan et al., 2019). It is important to note that unanticipated toxicity in animal or early-phase clinical studies has halted the clinical development of other compounds. Almost all available G12Ci have a selectivity threshold in preclinical studies, i.e., a concentration above which they also inhibit the growth of KRAS

wild-type cells. The covalent nature of drug binding may lead to off-target effects caused by non-selective interaction with cysteine residues in other proteins. Global proteomic analyses aiming to identify all cysteine residues that are covalently-modified by the drug (first described by Patricelli et al., 2016) show a clear selectivity for KRAS(G12C) in cells harboring this mutation. Were such studies to also be performed in wild-type KRAS cells, a setting where KRAS(G12C) cannot outcompete weaker targets, they would perhaps more accurately predict off-target effects in normal tissue and help understand the sources of G12Ci-toxicity. Factors beyond covalent-binding may also contribute to toxicity, especially considering that the severity of toxicity varies between drugs.

Efficacy data are beginning to emerge from the clinical trial investigating sotorasib, the first G12Ci to advance past dose-escalation phase. Of the lung cancer patients treated with sotorasib, nearly half had at least a partial response at initial evaluation (Govindan et al., 2019). Only a subset of these responses (~30%) were confirmed on subsequent evaluation, suggesting that early adaptation limits the effect of therapy. The vast majority of colorectal cancer patients did not have radiologic response to treatment, although considerable symptomatic improvement has been reported. MRTX849 appears to have similar effects in lung and colorectal cancer patients, yet more mature data are needed to validate early results (Christensen et al., 2019). While similar in inhibitory mechanism, sotorasib and MRTX849 differ in their elimination half-lives (sotorasib: 6h and MRTX849: 25h) and administration schemes (with sotorasib dosed daily and MRTX849 twice daily).

The lack of a G12Ci-treatment response in most patients with colorectal cancer (and in some patients with lung cancer) invokes studies suggesting that not all KRAS-mutant cancers are dependent on this oncoprotein for growth (Singh et al., 2012). Alternatively, it may be that the ability to target the oncoprotein in some settings is compromised. For example, BRAF V600-mutant colorectal cancers are insensitive to RAFi-treatment (Corcoran et al., 2012; Prahallad et al., 2012), owing to a higher activation of EGFR and the formation of RAFi-insensitive RAF dimers (Poulikakos et al., 2010). These tumors retain dependency on BRAF and targeting multiple nodes of the pathway results in better antitumor effects (Kopetz et al., 2020). A conclusion that cancers are independent of KRAS(G12C) thus requires evidence of potent and sustained target inhibition without an effect on phenotypes associated with a selective growth-advantage.

Adaptation to treatment

G12Ci-treatment only briefly suppresses KRAS signaling in cells (Janes et al., 2018; Lito et al., 2016; Misale et al., 2019; Ryan et al., 2019). Within 24-72h of treatment, an initial oncoprotein signaling inhibition is accompanied by a re-accumulation of active KRAS and a reactivation of ERK signaling (Xue et al., 2020). This pattern is consistent with adaptation, a process that is well-described in response to RAF or MEK inhibitors and one that limits their therapeutic potential (Lamba et al., 2014; Lito et al., 2012; Lito et al., 2014; Manchado et al., 2016; Montero-Conde et al., 2013; Sun et al., 2014). The accumulation of active KRAS suggests that a compensatory activation of receptor tyrosine kinases (RTK) and SOS1/2, which are both feedback suppressed by ERK, is in large part responsible for the adaptive changes noted during G12Ci-treatment. However, inhibition of ERK also leads to down-

regulated expression of MAPK phosphatases (e.g., DUSP4, DUSP6 etc.), which in turn results in increased ERK activation (Eblaghie et al., 2003). Although, in principle, this effect may also contribute to G12Ci adaptation, it has not yet been experimentally validated.

RTKs may modulate adaptation to G12Ci-treatment in two ways (Figure 2). By stimulating SOS1/2-mediated nucleotide exchange, RTK activation shifts KRAS(G12C) to its GTP-bound conformation, which is insensitive to the drug. RTKs can also bypass inhibition in a G12C-independent manner, i.e., through the activation of wild-type RAS, PI3K/AKT/mTOR or other pathways (Janes et al., 2018; Misale et al., 2019; Ryan et al., 2019). The role of PI3K/mTOR is supported by the presence of antiproliferative synergy when the G12Ci is combined with PI3K or mTOR inhibitors (Hallin et al., 2020; Misale et al., 2019). While it is evident that wild-type RAS proteins are feedback activated following G12Ci treatment, it is not clear whether this represents a compensatory response or one that drives adaptation. To this end, further testing is needed to show that wild-type RAS down-regulation enhances G12C inhibition and to determine the relative contribution of NRAS, HRAS and/or wild type KRAS (in cells harboring a heterozygous KRAS(G12C) mutation).

The adaptive signaling changes noted above have been integrated with single-cell modeling to show that adaptation to G12Ci-treatment occurs rapidly and in a non-uniform manner across cancer cell populations (Xue et al., 2020). Shortly after treatment, some cells are sequestered in a quiescent (G0) state with low KRAS activity, whereas others bypass this effect to resume proliferation. The divergent response is modulated by the production of new KRAS(G12C) and its distribution between the active/drug-insensitive and inactive/drug-sensitive states. New KRAS(G12C) is synthesized in response to suppressed ERK signaling and it is more susceptible to activation by nucleotide-exchange than baseline KRAS(G12C), which signals in the presence of feedback-suppressed SOS1/2. This explains why G12Ci-rechallenge is less effective than initial treatment and how active KRAS can re-accumulate during G12Ci-treatment, even though the drug binds in covalent/irreversible manner. Taken together these findings suggest that sustained inactive state-selective KRAS(G12C) inhibition can be achieved by blocking the production of new KRAS, enhancing its degradation or preventing its conversion to the active/drug-insensitive state.

Acquired resistance

Acquired resistance limits the clinical benefit of almost all targeted therapies and it is highly likely to also limit the effect of G12Ci. Although models with acquired resistance have not yet been reported, the mechanisms of inhibition and adaptation make several predictions on how acquired resistance to G12Ci-treatment might emerge.

Events predicted to attenuate target inhibition include: a) Secondary mutations on KRAS(G12C) that impede drug binding or enhance the propensity for the active/drug-insensitive conformation. b) Genetic events that consolidate the up-regulation of KRAS, such as KRAS amplification (Sale et al., 2019; Wong et al., 2018) or alterations leading to its diminished degradation (Bigenzahn et al., 2018). c) Activation of nucleotide-exchange through alterations in GEFs, like SOS1/2, or other upstream intermediates, such as RTKs or SHP2.

Events predicted to mediate resistance while the target remains inhibited, include: a) Activation of downstream signaling through gain-of-function events in RAF, MEK or ERK as well as loss-of-function events in RB1 or cyclin-dependent kinase inhibitors (e.g., p21, p27). SHP2. b) Activation of parallel signaling pathways through loss of NF1 or PTEN or activating mutations in other RAS family GTPases.

Studies investigating the effects of genetic KRAS down-regulation and those describing resistance to MEKi treatment provide additional insights into events that may lead to G12Ci-resistance. These include epithelial-to-mesenchymal transformation, through ZEB1 or YAP1 (Peng et al., 2019; Shao et al., 2014) as well as alterations in metabolic dependencies, including glycolysis (Amendola et al., 2019; Kerr et al., 2016), glutaminolysis (Romero et al., 2017, macropinocytosis (Commisso et al., 2013) and autophagy (Bryant et al., 2019; Kinsey et al., 2019).

Maximizing therapeutic impact

Clinically-active KRAS(G12C) inhibitors have a tremendous potential for impacting patient care, especially in patients with lung cancer, a disease affecting approximately 230,000 people each year in the US. Below we present several approaches aimed at maximizing their clinical benefit.

Optimizing monotherapy.

Drug-exposure directly correlates with the magnitude of ERK inhibition and response in patients with BRAF(V600) mutant tumors treated with a RAFi (Bollag et al., 2010). In this setting, a greater than 80% inhibition of ERK phosphorylation is needed to achieve clinical responses. Thus, efforts to establish inhibitors with an even higher affinity for the inactive-state of KRAS(G12C) will ensure a more potent and durable inhibition by delaying adaptation. Compounds that directly target active KRAS(G12C), such as the ‘tricomplex’ active state-selective inhibitors described above, also hold promise in this regard (provided that they retain a therapeutic index and can be translated to clinical trials).

Where feasible, alternative formulations, or administration schemes, enabling high drug exposures in patients may enhance the response rate and/or its magnitude. For example, very few adverse-effects leading to drug-discontinuation were observed during dose-escalation for sotorasib. It will be interesting to determine if sotorasib produces stronger antitumor effects when administered to patients on a twice-daily schedule.

Intermittent or pulsatile therapy has been suggested to prolong the response to inhibition of BCR-ABL, EGFR or BRAF (Das Thakur et al., 2013; Shah et al., 2008), to enable multimodal pathway inhibition (Xue et al., 2017) and to improve antitumor immunity (Choi et al., 2019). Intermittent KRAS(G12C) inhibition may have merit in principle, but the expected benefits need to be leveraged against the irreversible nature of drug-binding. More experimentation is required to determine the duration of target-engagement in wash-out studies and if intermittent dosing mitigates the adaptive changes noted above.

Pharmacodynamic endpoints must be implemented in the clinical assessment of the G12Ci-treatment response. Irreversible drug-binding affords the direct quantification of drug-bound KRAS(G12C) in tumor biopsies. Coupled with standard approaches to measure the phosphorylation of ERK or other KRAS signaling intermediates, this can provide invaluable clinical insight. Non-invasive cell-free DNA assays offer an alternative approach to estimate the effect of treatment on KRAS(G12C) allele burden over time. Such interventions are particularly important if we are to distinguish tumors that grow independently of KRAS(G12C) from those where KRAS(G12C) is not susceptible to inactive state-selective inhibition. They can also help understand the determinants of the transient response in some lung cancer patients.

Rational drug combinations.

Owing to a wide therapeutic-index, G12Ci are well-suited for combination therapy. Emerging paradigms include co-targeting upstream, downstream or parallel signaling pathways, as well as cell-cycle or immune checkpoints (Figure 2).

Co-*targeting upstream signaling*.

Suppressing nucleotide-exchange increases the residency of KRAS(G12C) in its inactive/drug-sensitive state and enhances the therapeutic effect of G12Ci (Figure 1C). Since RTK activation is a key stimulus for exchange, the first invocation of this idea was in experiments demonstrating enhanced antiproliferative effects by co-targeting RTKs (Lito et al., 2016; Patricelli et al., 2016). RTKi directly increase the ability of the G12Ci to engage its target (Patricelli et al., 2016). However, the ‘dominant’ RTK that drives GTP-loading of KRAS varies across models (Lito et al., 2016) and to be effective, this approach requires an *a priori* knowledge of which RTK to target. Not surprisingly, EGFR has emerged as an early lead and G12Ci combinations with cetuximab or erlotinib are entering clinical testing in patients with colorectal (Amodio et al., 2020) or lung cancer, respectively.

Direct suppression of nucleotide-exchange downstream of multiple RTKs is also effective, as evidenced by using SOS1-specific siRNAs (Lito et al., 2016) or emerging SOS1-selective inhibitors, such as BAY293 or BI1701963 (Hillig et al., 2019; Hofmann et al., 2019). The G12Ci/SOS1 combination is an attractive candidate for clinical testing; yet it remains to be seen if compensatory activity by SOS2, or other RAS-specific GEFs, hinders selective SOS1 inhibition.

An alternative approach is to co-target SHP2, an adaptor phosphatase which, among other functions, has also been implicated in the RTK-dependent activation of SOS1/2 (Fedele et al., 2018). Several studies demonstrate that SHP2i (such as RMC4630 and TNO155) enhance the antiproliferative and antitumor effects of G12Ci (Hallin et al., 2020; Lou et al., 2019; Ryan et al., 2019; Xue et al., 2020). On the basis of these findings clinical trials evaluating the benefit of MRTX849/TNO155 or sotorasib/RMC4630 are imminent.

Co-*targeting parallel signaling*.

While G12Ci-treatment potently inhibits RAF/MEK/ERK, the effect on PI3K/AKT/mTOR signaling is more subtle. Since the activation of PI3K requires input from RAS and RTKs,

both signals may need to be inhibited for complete AKT blockade in KRAS(G12C) mutant cells. Furthermore, models wherein pERK inhibition is coupled with suppressed pS6K/pS6 exhibit a more pronounced antiproliferative effect, as compared to those with pERK inhibition alone (Janes et al., 2018; Misale et al., 2019). S6K is a known substrate of mTOR, which is activated downstream of AKT, but it can also be activated by RSK, downstream of ERK. Despite the unclear mechanism of PI3K, AKT and mTOR activation in KRAS(G12C) mutant cells, the combined inhibition of PI3K or mTOR alongside KRAS(G12C) has more pronounced antitumor effects than either drug alone (Hallin et al., 2020; Misale et al., 2019). Similarly, a three-drug combination targeting KRAS(G12C), IGF1R and mTOR led to potent antitumor effect (Molina-Arcas et al., 2019), supporting RTK/PI3K/AKT/mTOR as a target pathway for combination therapy.

Co-targeting downstream signaling.

An intuitive approach to enhance KRAS(G12C) inhibition is by targeting RAF dimers, MEK or ERK. However, inhibition of ERK output releases the feedback suppression of SOS1/2, which may impede target engagement by the drug. The MEKi trametinib does not significantly enhance the effect of ARS1620 or MRTX849 but its reported to enhance the antitumor effect of sotorasib (Canon et al., 2019). The sotorasib/trametinib combination is now in clinical testing and data from this trial will inform the potential benefit of co-targeting RAF/MEK/ERK alongside KRAS(G12C).

Co-targeting cell-cycle checkpoints.

A key output of KRAS signaling is the activation of the CyclinD:CDK4/6 leading to RB1 hyper-phosphorylation and progression through the G1/S cell-cycle checkpoint. G12Ci-treatment sequesters cells in a quiescent (G0) state, with adapting cells progressing through the G1/S and G2/M checkpoints. Two approaches that enhance the effect of G12Ci by maximizing cell-cycle arrest, include targeting CDK4/6 with palbociclib (Hallin et al., 2020; Lou et al., 2019) or AURKA with alisertib (Xue et al., 2020).

Co-targeting immune checkpoints.

KRAS signaling has an immunosuppressive effect on the tumor microenvironment (Coelho et al., 2017; Pylayeva-Gupta et al., 2012; Ruscetti et al., 2020). KRAS(G12C) inhibition might therefore enhance immune checkpoint inhibition, a hypothesis tested in an immune-competent colorectal cancer model (Canon et al., 2019). Indeed, sotorasib enhanced the expression of inflammatory chemokines leading to enhanced T cells infiltration. When combined with a programmed cell-death protein 1 (PD1) antibody, sotorasib led to a complete and durable antitumor effect. More work is needed to understand how KRAS(G12C) leads to immunosuppression, an effort that will be aided by the generation of immune-competent KRAS(G12C) mutant mice (Li et al., 2018b; Zafra et al., 2019). Perhaps even more informative will be the results from an ongoing clinical trial testing the benefit of sotorasib in combination with PD1 blockade in patients.

Summary and future directions

The last seven years have seen tremendous progress leading to clinically-active inhibitors and a better understanding of how KRAS oncoproteins signal in cancer. We now recognize that KRAS(G12C), one of the most common driver oncoproteins in lung cancer, exists in an excitable state and undergoes nucleotide-cycling between its active and inactive conformations in cancer cells. As a consequence, it is dependent on nucleotide-exchange for activation and susceptible to drugs that block this process. Inactive state-selective inhibitors disrupt this cycle and trap KRAS(G12C) in its GDP-bound state to suppress tumor growth in cancer patients.

How does KRAS(G12C) hydrolyze sufficient GTP to enable cycling in cancer cells? The consensus view from recent reviews suggests that KRAS(G12C) is unique among KRAS oncoproteins, in that it has a high intrinsic GTP hydrolysis rate. An alternative possibility is that GTP-hydrolysis by KRAS mutants is subject to regulation by yet-to-be-determined cellular factors. If true, the latter would suggest that KRAS oncoproteins are broadly susceptible to inactive state-selective inhibition (provided the identification of chemical scaffolds that engage KRAS oncoproteins in a non-cysteine dependent manner).

The duration of KRAS(G12C) inhibition is limited by adaptation, a process that may be responsible for the less than maximal therapeutic effects observed in clinical trials. Other clinically relevant modulators of therapeutic response, including mechanisms of acquired resistance, tissue specific dependencies or the effect of alterations that co-occur alongside KRAS(G12C), are still under investigation. Nevertheless, emerging combination therapies that ensure maximal and durable inhibition of KRAS(G12C) signaling, along with those that enhance the effect of the immune system, are currently being translated into the clinic and are highly likely to enhance the therapeutic benefit of KRAS(G12C) inhibition.

The success of inactive state-selective KRAS(G12C) inhibitors is paving the way for inhibitors with broader therapeutic utility. These include inactive state-selective inhibitors targeting non-G12C mutants, active-state-selective G12C inhibitors as well as compounds that prevent the membrane localization or induce the degradation of KRAS oncoproteins. In theory, inactive state-selective non-G12C inhibitors will likely be susceptible to adaptive mechanisms similar to those described for sotorasib, MRTX849 and ARS1620. Active-state-selective drugs, exemplified by the ‘tri-complex’ KRAS(G12C)-GTP:CYPA inhibitors, have potential advantages. By directly binding to and preventing effector interaction with active KRAS they are predicted to withstand some of the adaptive pressures that limit the effect of inactive state-selective compounds. However, the dependency on CYPA for inhibition may pose its own limitations that need to be addressed experimentally. Lastly, the cellular effects of degraders will depend on which KRAS conformation is targeted by the compound. Early KRAS degraders couple chemical moieties that stimulate degradation with warheads that engage G12C in a covalent manner and bind only to its inactive state.

Whatever their strengths or limitations, emerging KRAS-directed therapies will undoubtedly enrich our understanding of KRAS oncoprotein biology. At the same time, they hold promise in providing much needed therapeutic options for patients with KRAS mutant

cancers. In order to maximize the therapeutic potential of these exciting new therapies, preclinical evidence must be coupled with appropriate clinical trial designs, incorporating pharmacodynamic endpoints and evidence-based administration schemes. With optimal integration of basic, translational and clinical research activities, our collective efforts have the potential to bring forth unprecedented success in precision oncology.

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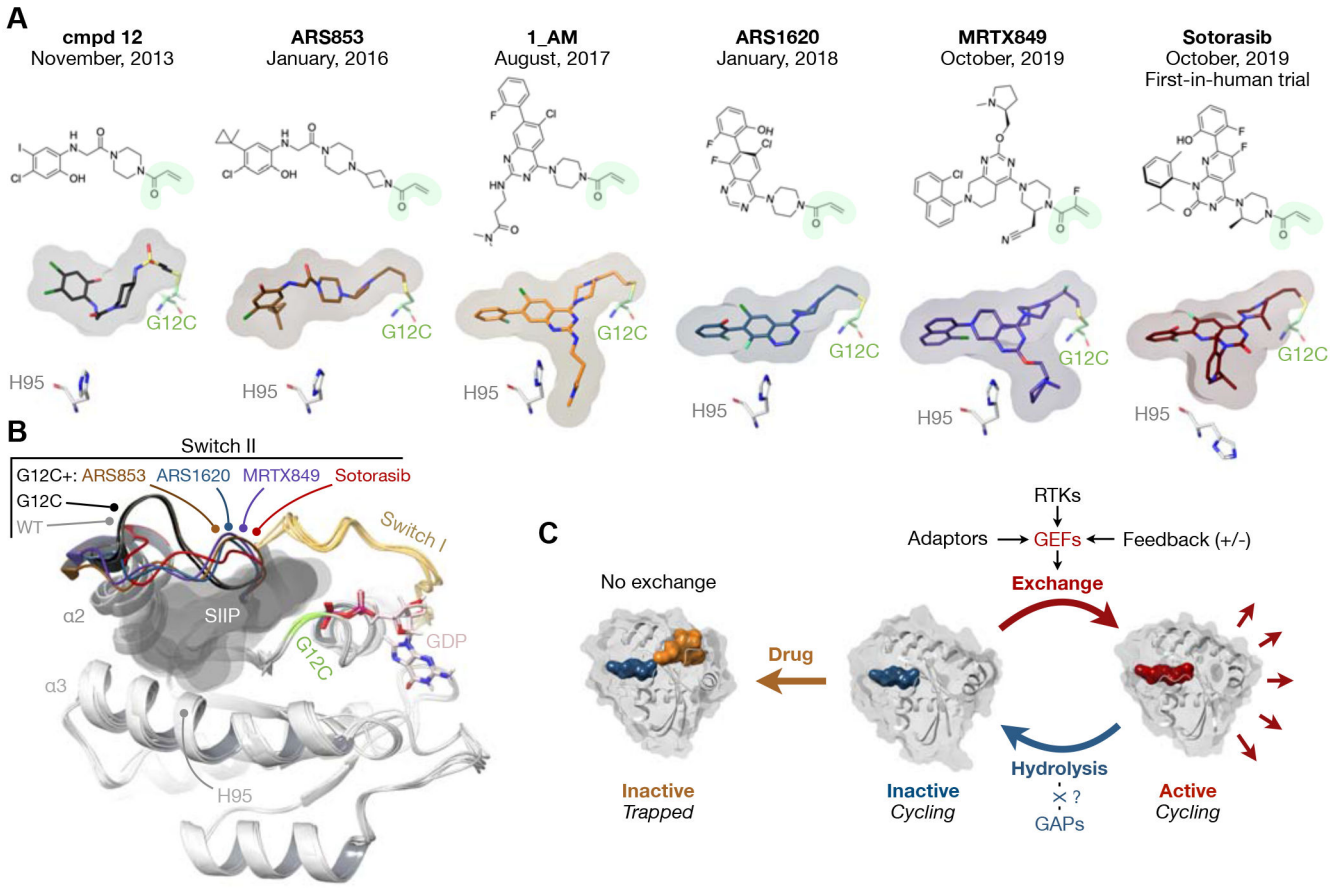

Figure 1. G12C inhibitors and their mechanism of action

A) Chemical structures of G12C inhibitors with their initial publication date. The surface plot denotes the orientation of each inhibitor in the SIIP of mutant KRAS. The acrylamide warhead that engages the G12C residue is shown in green. The orientation of the histidine (H) 95 residue on KRAS is also shown. An interaction with this residue is thought to enhance the potency of inhibition.

B) Superimposed structures of KRAS(G12C) bound to GDP alone, or in the presence of the indicated inhibitors. KRAS WT is included as a comparison. The surfaces in gray denote the area occupied by the inhibitors. Note the displacement of switch II in drug-bound structures. From 4LYF, 5F2E, 5V9L, 5V9U, 60IM, 6UT0, 4OBE and 4LDJ.

C) Inactive state-selective KRAS(G12C) inhibition requires intact GTPase activity and occurs by preventing nucleotide-exchange to the active state. KRAS oncoproteins are insensitive to GAPs (dotted line, see text) and a relatively high intrinsic hydrolysis rate by KRAS(G12C) is thought to be sufficient for inhibition. The alternative possibility is that GTP hydrolysis by KRAS oncoproteins is aided by unidentified cellular factors (question mark). Space-filling models of GTP, GDP and G12Ci are respectively colored in red, blue and orange.

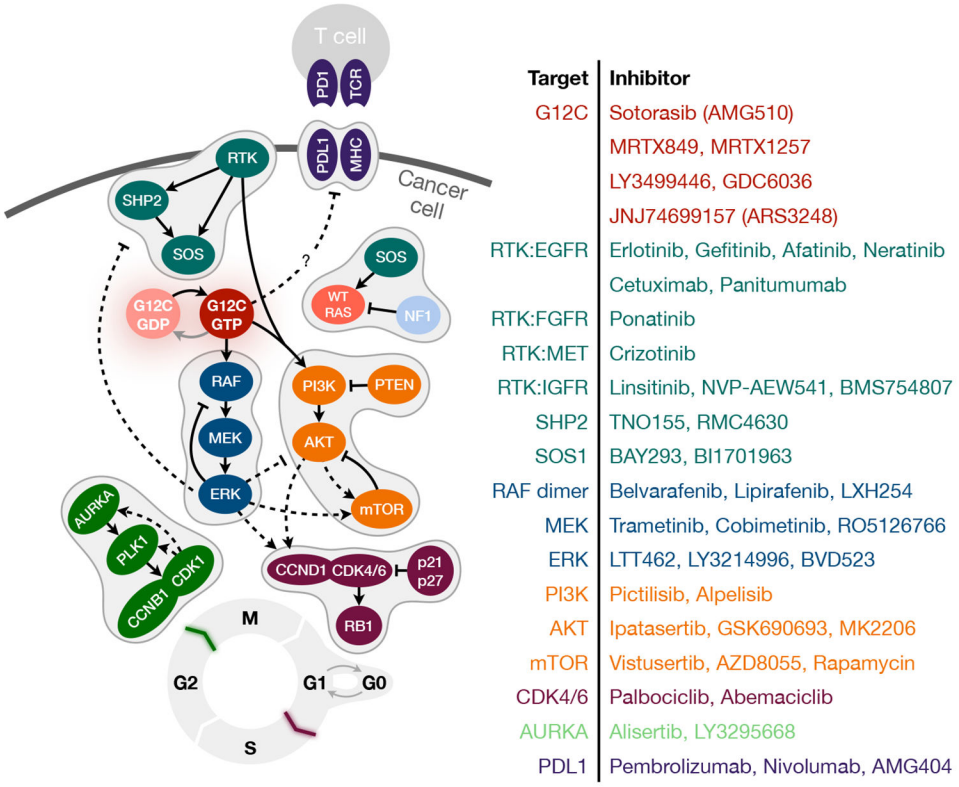

Figure 2. Opportunities for combination therapy

A simplified schematic of KRAS signaling along with inhibitors targeting various intermediates reported to modulate KRAS(G12C) inhibition. Solid lines indicate a direct effect whereas dotted lines indicate indirect effects. Colors denote signaling intermediates along the same pathway.

Table I.

KRAS(G12C) inhibitors in clinical trials

Drug	Status	Phase	Design	Trial
Sotorasib (AMG510)	Recruiting	I/II/III	Monotherapy or combination with: AMG404 (anti-PDl) Panitumumab RMC4630 Trametinib Afatinib	NCT04380753 NCT03600883 NCT04303780 NCT04185883
MRTX849	Recruiting	I/II	Monotherapy or combination with: Pembrolizumab Cetuximab Afatinib TNO155	NCT03785249 NCT04330664
GDC6036	Recruiting	I	Monotherapy	NCT04449874
LY3499446	Not recruiting *	I	Monotherapy	NCT04165031
JNJ74699157	Not recruiting *	I	Monotherapy	NCT04006301

* Terminated due to toxicity.