Therapeutic strategies: Targeting *KRAS* in cancer
The role of *KRAS* in cancer
Cancer’s ‘big four’

- **β-catenin**
  - “Needs its own destruction complex”

- **p53**
  - “The guardian of the genome”

- **KRAS**
  - “The beating heart of cancer”

- **MYC**
  - “The master regulator”

**KRAS is the most common RAS oncogene found in human cancer**

- RAS mutations were first reported in cancer over 30 years ago, and have since been validated in numerous studies as **drivers of tumor initiation** and maintenance\(^1\).

- In humans, **three RAS genes** encode a highly homologous subfamily of small GTP binding proteins: KRAS, NRAS and HRAS\(^2\).

- Of the three, **KRAS is the most frequently mutated**, constituting 86% of all RAS mutations\(^2\).

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### Frequency of RAS isoform mutations in human cancers\(^2\)

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Percentage of Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas</td>
<td>90%</td>
</tr>
<tr>
<td>Colon</td>
<td>42%</td>
</tr>
<tr>
<td>Small intestine</td>
<td>35%</td>
</tr>
<tr>
<td>Biliary tract</td>
<td>28%</td>
</tr>
<tr>
<td>Endometrium</td>
<td>22%</td>
</tr>
<tr>
<td>Lung</td>
<td>20%</td>
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<tr>
<td>Skin (melanoma)</td>
<td>20%</td>
</tr>
<tr>
<td>Cervix</td>
<td>19%</td>
</tr>
<tr>
<td>Urinary tract</td>
<td>16%</td>
</tr>
</tbody>
</table>

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GTP, guanosine triphosphate.

KRAS activity regulates multiple pathways involved in cell growth, proliferation and survival

- RAS GTPases cycle between an inactive, GDP-bound state and an active, GTP-bound state:\n  - GEFs (such as SOS1) help promote RAS activation
  - GAPs inactivate RAS by catalyzing GTP hydrolysis

- Activated, GTP-bound RAS binds to downstream RBD-containing effectors:\n  - There are at least 11 different RAS effector families that activate distinct signaling cascades
  - Preclinical and in vivo data support the driving role of four families (RAF, PI3K, RalGEF and TIAM1) in RAS-driven oncogenesis
  - Of these, MAPK signaling and (to a lesser extent) PI3K signaling appear to be the most important effectors downstream of KRAS.

ERK, extracellular regulated kinase; GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor; GDP, guanosine diphosphate; GTP, guanosine triphosphate; GTPase; guanosine triphosphatase; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; Pi, phosphate; PI3K, phosphoinositide 3-kinase; PIP₂, phosphatidylinositol-(4,5)-bisphosphate; PIP₃, phosphatidylinositol-(3,4,5)-trisphosphate; RA, RAS-association domain; RalGEF, Ral guanine nucleotide exchange factor; RBD, RAS-binding domain; SOS1, son of sevenless homolog 1; TIAM1, T-cell lymphoma invasion and metastasis-inducing protein 1.

**KRAS** mutations occur most frequently at one of three hotspots

- 98% of **KRAS** gain-of-function mutations occur at one of three hotspots (G12, G13 and Q61)
- The mutation profile varies according to tumor type:

![ KRAS mutation rates in PDAC

- G12: 97%
- G12C: 3%
- G13: 1%
- Q61: 2%

![ KRAS mutation rates in CRC

- G12: 78%
- G12C: 4%
- G13: 20%
- Q61: 1%
- Other: 1%

![ KRAS mutation rates in lung cancer

- G12: 89%
- G12C: 39%
- G13: 5%
- Q61: 3%
- Other: 3%

- The prognostic significance of **KRAS** G12C mutations varies depending on tumor type:
  - In patients with an oncogenic **KRAS** mutation, G12C is associated with longer PFS than other **KRAS** mutations in advanced NSCLC, and a poorer prognosis in primary CRC

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*KRAS* missense mutations (n=151) in primary lung adenocarcinoma.
CRC, colorectal cancer; NSCLC, non-small cell lung cancer; PDAC, pancreatic ductal adenocarcinoma; PFS, progression-free survival.

Oncogenic KRAS mutations result in impaired GTPase activity

- Oncogenic missense mutations **disrupt intrinsic GTPase activity**, resulting in a longer time spent in the active, GTP-bound state\(^1,2\)
  - Mutation of G12 and G13 interferes with GAP binding and GAP-stimulated GTP hydrolysis
  - Mutation of Q61 directly interferes with the catalysis of GTP hydrolysis
  - All three residues are involved with binding downstream effectors such as RAF

- Specific amino acid substitutions at these (and other) locations may have differential effects on response to therapy and patient outcomes\(^1\)

Figure adapted from Chen CC, et al. 2013.\(^3\)

GAP, GTPase-activating protein; GDP, guanosine diphosphate; GTP, guanosine triphosphate; GTPase; guanosine triphosphatase.

Targeting \textit{KRAS} in cancer
Therapeutic approaches targeting oncogenic KRAS activity may be direct or indirect

- **Direct approaches** aim to inhibit KRAS GTPase activity

- **Indirect approaches** aim to disrupt the effects of KRAS activity by targeting downstream targets or other related pathways

GTPase; guanosine triphosphatase.

In the past, KRAS has been considered to be an ‘undruggable’ target\(^1,^2\)

- By analogy to the successful development of clinically effective ATP-competitive inhibitors (e.g. tyrosine kinase inhibitors), small-molecule GTP antagonists should provide a straightforward strategy to target mutant KRAS\(^1\)

- However, this approach has been challenging due to the **picomolar affinity of RAS for GTP**, and the millimolar concentration of GTP within cells\(^1\)

- The surface topology of RAS (the **lack of well-defined hydrophobic surface pockets**) is not easily amenable to the design of high-affinity small-molecule antagonists\(^1,^3\)

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Figure adapted from Kessler D, et al.\(^4\)

ATP, adenosine triphosphate; GTP, guanosine triphosphate.

KRAS inhibition is being re-evaluated as a therapeutic strategy in a variety of cancers

• The KRAS G12C mutation creates a **pocket that may be exploited** by covalent inhibitors specific to this mutant protein\(^1\)\(^-\)\(^4\)
  – KRAS G12C inhibitors are of clinical interest in patients with lung adenocarcinoma, CRC and other cancers\(^5\)\(^,\)\(^6\)

• Multivalent inhibitors may be effective against multiple RAS proteins\(^7\)

• Other small-molecule inhibitors have been designed, which bind to a pocket present in both **active and inactive forms** of RAS\(^8\)
  – Blocking all GEF, GAP and effector interactions with KRAS results in inhibition of downstream signaling and an antiproliferative effect in KRAS mutant cells\(^8\)

Proof of concept: development of a covalently bound inhibitor against KRAS G12C\(^2\)\(^,\)\(^9\)

Protein pocket in KRAS G12D exploited by a small-molecule inhibitor\(^8\)

Figures adapted from Ostrem JM, et al.,\(^2\) Chen CC, et al.,\(^9\) and Kessler D, et al.,\(^8\)

CRC, colorectal cancer; GAP, GTPase-activating protein; GDP, guanosine diphosphate; GEF, guanine nucleotide exchange factor; GTPase, guanosine triphosphatase.

Disrupting the interaction between KRAS and SOS1 is a potential therapeutic strategy

- **SOS1 is a GEF** that regulates the KRAS GDP–GTP cycle by promoting nucleotide exchange, resulting in the formation of ‘active’ KRAS–GTP\(^1\)
  
- In contrast to KRAS G12C inhibitors, a **SOS1::KRAS interaction inhibitor** may be effective **regardless of KRAS mutation status**\(^1\)
  - Protein::protein interaction inhibitors are designed to bind to SOS1, preventing its interaction with KRAS–GDP

- As a result, KRAS is locked in its GDP-bound ‘off’ state; formation of **active, GTP-bound KRAS** is prevented\(^2\)

- In RAS-driven tumor cells, SOS1 inhibition **prevents activation of downstream signaling pathways**, and antagonizes the negative feedback mechanism induced by inhibition of the RAF/MEK/ERK pathway\(^2\)

ERK, extracellular regulated kinase; GDP, guanosine diphosphate; GEF, guanine nucleotide exchange factor; GTP, guanosine triphosphate; MEK, mitogen-activated extracellular signal regulated kinase; SOS1, son of sevenless homolog.

Inhibiting the RAS/RAF/MEK/ERK pathway at multiple levels could improve therapeutic efficacy

- Preclinical data suggest that the oncogenic effects of KRAS mutation may be mediated primarily by MEK activity.\(^1\)

- However, to date, MEK1/2-selective inhibitors have not shown potent activity in RAS-mutant cancers, due to:
  - Vertical compensation mechanisms that lead to reactivation of ERK via upregulation of upstream pathway components
  - Compensatory activation of the PI3K/AKT/mTOR pathway

- Combining a SOS1::KRAS inhibitor with a MEK inhibitor could result in a more robust pathway blockade than either compound alone, resulting in a prolonged antitumor response that is less vulnerable to compensatory mechanisms or acquired resistance.\(^2,3\)

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ERK, extracellular regulated kinase; GDP, guanosine diphosphate; GTP, guanosine triphosphate; PI3K, phosphoinositide 3-kinase; MEK, mitogen-activated extracellular signal regulated kinase; mTOR, mammalian target of rapamycin; SOS1, son of sevenless homolog 1.

Preventing KRAS membrane association has not been a clinically effective strategy

- RAS proteins undergo post-translational modifications to enable membrane association, a key step in their activation\(^1,2\)
- **Farnesylation** was shown to be required for KRAS membrane association and oncogenic signaling, and was, therefore, considered a promising therapeutic strategy in RAS-driven tumors\(^1,2\)
- Despite early preclinical data suggesting that farnesyltransferase inhibitors could be effective, they failed to show efficacy in the clinic:\(^1,2,4\)
  - Although HRAS is solely dependent on farnesyltransferase for membrane association, KRAS and NRAS may also be alternatively prenylated by geranylgeranyltransferase
  - Many endogenous proteins depend on farnesylation, raising toxicity concerns related to off-target effects
- **Renewed interest** in farnesyltransferase inhibitors has been triggered by initial signs of activity in HRAS-mutant head and neck tumors\(^5\)

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**Figure adapted from Chen C-C, et al. 2013.**

**Post-translational prenylation of KRAS\(^1-3\)**

- **C-terminal hypervariable region**
  - Farnesyltransferase
  - Geranylgeranyltransferases I and II

**Membrane association and activation**

- Switch I
- Switch II
- GTP
- P-loop
- C-terminal hypervariable region
- R79/Gap
- P-loop
- Switch I
- Switch II
- Q61
- F32
- R63
- V64
- V32
- C-terminal hypervariable region
Exploiting KRAS-dependent metabolic pathways is still at an early stage

- Oncogenic KRAS activity promotes metabolic reprogramming of tumor cells, supporting continued growth and proliferation through the use of a variety of intracellular and extracellular metabolite sources.

- These adaptations result in tumor-specific metabolic vulnerabilities, due to reliance on pathways including:
  - Macropinocytosis
  - Autophagy
  - Glucose uptake/metabolism
  - Pentose phosphate metabolism
  - Glutamine metabolism (redox homeostasis)
  - Lipid homeostasis

- Inhibition of these pathways could provide therapeutic opportunities that are selective for KRAS-driven tumor cells, but further preclinical research is needed before these can be investigated in the clinic.

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**EN01, enolase 1; GFPT1, glucosamine–fructose-6-phosphate aminotransferase isomerising; GLUD1, glutamate dehydrogenase 1; GLUT1, glucose transporter 1; GOT1/2, glutamic-oxaloacetic transaminase 1/2; HK1/2, hexokinase 1/2; LDHA, lactate dehydrogenase A; NADP+/NADPH, nicotinamide adenine dinucleotide phosphate; PEP, phosphoenolpyruvate; PFK1, phosphofructokinase 1; ROS, reactive oxygen species; TCA, tricarboxylic acid.**

Targeting synthetic lethality in KRAS mutant cancers is not yet feasible

- Synthetic lethality refers to an interaction between two co-essential genes: inhibiting both targets simultaneously can induce tumor cell death\(^1\)
  - E.g. PARP inhibitors in cancers deficient in the homologous recombination repair pathway\(^1\)

- Through screening, many synthetic lethal interactors with mutant KRAS have been identified. Essential pathways for survival include: co-operative signaling, transcriptional regulation and maintenance of genomic stability\(^1\)

- In preclinical studies, inhibition of SHP2 induces senescence in KRAS-mutant NSCLC (and is exacerbated by concomitant MEK inhibition)\(^1\)

- However, many such genes identified through screening are not currently druggable

- Also, synthetic lethality studies are typically based on KRAS-mutant cell lines or tumor models; due to the heterogeneity of such tumors *in vivo*, targeting synthetic lethality in the clinic remains an unproven strategy\(^1\)

ADP, adenosine diphosphate; NSCLC, non-small cell lung cancer; MEK, mitogen-activated protein kinase kinase; PARP, poly (ADP-ribose) polymerase; SHP2, SRC homology region 2-containing protein tyrosine phosphatase 2.

Summary
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• Mutations in RAS genes (KRAS, NRAS and HRAS) were first reported in cancer over 30 years ago and have since been validated as key drivers of tumor initiation and maintenance,\(^1\) firmly positioning RAS as one of the ‘big four’ in cancer

• KRAS is the most frequently mutated RAS oncogene, constituting 86% of all RAS mutations in human cancer\(^2\)

• 98% of KRAS mutations occur at one of three mutational hotspots (G12, G13 and Q61),\(^1\) resulting in impaired GTPase activity, upregulation of KRAS signaling, and downstream activation of pathways involved in tumor growth, proliferation and survival\(^1\)

GTP, guanosine triphosphate.

Summary (contd.)

- Although RAS has a long-standing reputation for being ‘undruggable’, recent advances in drug discovery and lead optimization have led to the development of KRAS inhibitors, as well as inhibitors of key upstream activators such as the SOS1::KRAS interaction inhibitor.
- Several KRAS-targeted compounds are now under investigation in clinical trials.
- Other therapeutic approaches targeting KRAS have failed to demonstrate clinical benefit (such as blocking membrane association); meanwhile, targeting metabolic dependencies of KRAS-driven tumors, or inhibiting synthetic lethal partners, will require further preclinical validation before investigation in the clinic.

SOS1, son of sevenless homolog 1.