Article Type: Review

**KRAS: reasons for optimism in lung cancer**

C.R. Lindsay¹,²,³, M. Jamal-Hanjani³,⁴, M. Forster³,⁴, F. Blackhall¹,²,³

1. Division of Molecular and Clinical Cancer Sciences, University of Manchester, Manchester, UK.
2. Department of Medical Oncology, The Christie NHS Foundation Trust, Wilmslow Road, Manchester, M20 4BX, UK.
4. Department of Oncology, University College of London Hospital and UCL Cancer Institute, London, UK.

**Corresponding Author:** Dr Colin Lindsay, Division of Molecular and Clinical Cancer Sciences, University of Manchester, Manchester, UK. Tel: +44 (0)161 446 3745.

colin.lindsay@manchester.ac.uk
Abstract

Despite being the most frequent gain-of-function genetic alteration in human cancer, KRAS mutation has to date offered only limited potential as a prognostic and predictive biomarker. Results from the phase III SELECT-1 trial in non-small cell lung cancer (NSCLC) recently added to a number of historical and more contemporary disappointments in targeting KRAS mutant disease, including farnesyl transferase inhibition and synthetic lethality partners such as STK33. This narrative review uses the context of these previous failures to demonstrate how the knowledge gained from these experiences can be used as a platform for exciting advances in NSCLC on the horizon. It now seems clear that mutational subtype (most commonly G12C) of individual mutations is of greater relevance than the categorical evaluation of KRAS mutation presence or otherwise. A number of direct small molecules targeted to these subtypes are in development and have shown promising biological activity, with some in the late stages of preclinical validation.

154 words
Key words: KRAS, NSCLC, RAS, non-small cell lung cancer, G12C
RAS is the most common oncogene in cancer, with its mutation occurring in approximately 30% of all cases [1]. This rate of mutation can vary substantially in different cancer types, with its most frequent rates of modification found to affect KRAS in pancreatic, colorectal and lung adenocarcinomas [2-4]. Its function and importance as a GTPase is evidenced by its central coordinating role in the cell, where it connects upstream signals from cell surface receptors such as FGFR, EGFR and ERBB2-4 to downstream cancer-associated pathways such as MAPK, PI3K and Ral [5]. Introduction of RAS mutations in mice using either chemical and/or environmental induction or genetic modification has been shown to induce cancers such as lung adenocarcinoma and melanoma, with perhaps the most well characterised lung cancer model demonstrating that lung-specific KRAS G12D expression can allow mediation of both tumor initiation and multiplicity [6-9]. More recent studies of tumor heterogeneity have identified RAS mutations as both ‘truncal’ and ‘branch’ drivers, depending on genetic subtype and the cancer cell context [10].

Predominant isoforms and genetic subtypes of mutant RAS vary with cancer histology. In terms of isoforms, NRAS is the most frequently mutated in melanoma, whereas KRAS mutation occurs most frequently in adenocarcinomas [11]. Mutant HRAS occurs in a small percentage of head and neck squamous cancers [12]. Why certain cancers are driven by specific RAS isoforms remains unknown, and it is also unclear whether this variation occurs as a consequence of cell lineage and/or other factors. In lung cancer, KRAS mutation occurs in 20-40% of adenocarcinomas, with codons 12 and 13 mutations being the most frequent, of which the most common subtypes are G12C, G12V and G12D. G12C and G12V have been epidemiologically associated with a smoking history, whereas the G12D subtype occurs more frequently in never smokers [13].

Following its description as a key cancer mutation in 1982, clinical investigation of RAS has been blighted by conflicting results regarding its prognostic relevance as well as unsuccessful clinical trial programmes, most recently evidenced by the large phase III SELECT-1 trial which showed no improvement in progression-free or overall survival with the addition of selumetinib, an oral small-
molecule MEK inhibitor, to 2nd line docetaxel chemotherapy in advanced KRAS-mutant NSCLC [14,15]. The context of this failed progress has been particularly disappointing: lung cancer is the most common cause of cancer-associated mortality in the UK and worldwide, with treatment for its most common histological category, NSCLC, offering only limited survival gains in both early and metastatic disease settings [16,17]. This translational review will argue that these past difficulties are by no means representative of KRAS irrelevance, but more a consequence of insufficient understanding of its biology coupled with sub-optimal study design. Here, strategies to improve delineation of the prognostic and predictive potential of KRAS as a biomarker will be presented, as well as reasons to be optimistic for the future success of KRAS targeting in lung cancer.

Biomarker challenges

Within phase I of the Cancer Research UK Stratified Medicine Programme, KRAS mutation was detected in 287 of 774 lung adenocarcinomas (37%), a figure which contrasts with a prior large American study reporting KRAS mutations in 21% of 482 adenocarcinomas [18]; these percentages can reasonably be assumed to be the upper and lower limits of normal for KRAS mutation incidence in NSCLC. KRAS mutations of codons 12 and/or 13 'not otherwise specified' were noted in 79 patients, leaving smoking-associated mutational subtypes to represent at least 56% of cases, most commonly G12C (73/208 samples, 35.1%) and G12V (44/208 samples, ~21.2%), while the G12D subtype (40/208 samples, ~19.2%) has been closely associated with never smokers [13]. The remaining cases can be constituted by a wide variety of mutational subtypes, involving usually codon 12 or codon 13 (Figure 1).
Clinical reports from the past 5 years have progressively demonstrated the importance of interrogating KRAS mutation beyond a simple categorisation of whether it is present or not [19,20]. Its historical study as a prognostic biomarker in lung cancer frequently concluded, in line with its important biological context, that its presence conferred diminished survival. Many of these reports were limited by small numbers of patients, retrospective data, and lack of a validation set and/or multivariate analysis, but subsequent meta-analyses offered similar conclusions [21,22]. However, an examination in 2013 by the LACE bio-collaborative group offered the most comprehensive single study assessment of this question with conflicting results. In greater than 1,500 KRAS-tested patients recruited from four key lung cancer adjuvant clinical trials, no clear prognostic or predictive relevance of KRAS mutation was observed. There was a suggestion that a small number of patients with codon 13 KRAS mutations did not benefit from adjuvant chemotherapy, however this could not be confirmed due to the lack of a validation set. Perhaps as important as its findings, this study offered the first analysis of KRAS prognostication according to mutational subtype – a genetic context that would shape prognostic and treatment progress in subsequent years [23].

So why would KRAS mutation succeed as a biomarker now when it has failed in the past? There are three particular reasons to be optimistic regarding the development of KRAS as a clinical biomarker and target in lung cancer:

1. **It is predictive.**

Cancer biomarkers with routine clinical utility are generally either predictive (such as BRAF mutation in melanoma or ALK/ROS-1 gene fusion in lung cancer), or have been more historically used for tumour monitoring (for example, CA125 in high grade serous ovarian carcinoma) [24-26]. KRAS mutation in lung adenocarcinoma has recently been characterised as a predictor of negative benefit for patients with EGFR mutant disease undergoing treatment with EGFR tyrosine kinase inhibitors, a
finding that mirrors similar predictive benefits of KRAS mutation in colorectal cancer [27,28]. With the anticipated influx of direct KRAS mutation inhibitors to the clinic (discussed below) over the next few years, the opportunity to exploit the predictive potential of KRAS mutation will undoubtedly assume increasing relevance in a manner that may be analogous to the successes achieved with BRAF targeting across cancer [29].

2. Improved characterisation of KRAS subtypes, amplification and genomic context.

More recent prognostic investigation of KRAS mutation at the level of its genetic subtypes in lung cancer has so far offered more conclusive results. Ihle and colleagues demonstrated reduced survival in patients with the more common smoking-related G12C and G12V subtypes, also showing that their pathogenicity was conferred by upregulation of downstream Ral and PI3K signalling [19]. This report was consistent with another study that retrospectively analysed over 800 patients with the G12V subtype, also concluding that this was associated with reduced prognosis [20]. Identification of KRAS mutation copy number gain and amplification in lung cancer has been reported in cancers from mouse models harbouring the G12D subtype, a change that was associated with increased disease aggression [30]: although traditionally considered to be a cancer driven by somatic mutation as a consequence of smoking and environmental insults, undoubtedly more will follow regarding the characterisation of lung adenocarcinoma as a cancer further defined by copy number change, as has been the case more recently with oesophageal and ovarian carcinomas [31,32]. This level of genetic examination, whether through assessment of genetic subtypes and/or copy number aberration, was omitted in historical biomarker studies of lung cancer and will be paramount to our future exploitation of KRAS as a lung cancer biomarker. Moreover, genetic background and context of each RAS-driven tumour will also assume further relevance, a complexity which has been one of a number of reasons cited for the lack of success observed so far with synthetic lethality screens in KRAS mutant disease [33]. One key report has categorised KRAS-mutant adenocarcinoma into three major
subgroups (co-mutation with aberrations in LKB1, TPS3 or CDKN2A/B), assisting characterisation of their relative immune profiles and therapeutic vulnerabilities [34].

3. There is a paradigm for routine KRAS testing in the clinic.

The backdrop for introduction of any new biomarker to the hospital setting has become significantly more accommodating in recent years - to the extent that cancer and lung cancer-specific organisations such as ASCO, ESMO, and the IASLC have issued position statements and guidelines on clinical molecular testing to be performed routinely on lung cancer patients. Targeted sequencing involving a panel of core cancer genes such as RAS is now possible at substantially reduced time and cost, with many molecular pathology departments across publicly-funded hospitals in the Western world incorporating this technology into their short to medium-term plans for implementation. Such a setup will foster optimisation of other biomarker test qualities stipulated in REMARK guidelines: an increase in reproducibility allied to a reduction of complexity [35]. A number of academically led lung cancer stratified-medicine programmes rolled out in the US, UK and Europe will have a key role in informing this process, and the example of lung cancer molecular testing implemented across France has set a standard for others to follow [36].

Why has there been failure to date to effectively target KRAS in NSCLC?

The structural and biochemical challenges of targeting RAS have held back a research environment which has been characterised for a number of years by its lack of a breakthrough. KRAS in particular has been coined as an ‘undruggable’ target when in reality structural biochemists and drug developers have considered it to be challenging but possible. The recent success of targeting BCL-2 (another ‘undruggable’ target) has highlighted how progress can be made with difficult targets by
applying an emerging understanding of structural biochemistry characterised through an increased success of NMR and X-Ray crystallography [37].

Several lessons have been learned in recent years that represent challenges for effective KRAS targeting. First, it binds to GTP with nanomolar affinity in its active state, an interaction that requires more potent inhibition than that conferred by traditional tyrosine kinase inhibitors. Second, it is structurally a relatively smooth protein, with few clefts for potential lead compounds to ‘hook’ on to in order to exert their effects [1]. As a consequence, KRAS mutation targeting has been focused for many years on downstream inhibitors and, more recently, targeting of identified synthetic lethality partners. For different reasons, neither approach has yet offered significant successes:

1. Downstream inhibition.

Farnesyl transferase inhibitors (FTIs) offer the most recited example of treatment failure in targeting RAS mutant cancer. Preclinical research had suggested their potential efficacy through inhibition of membrane association by mutant Ras and its subsequent activation (mediated by farnesyl transferase), yet clinical trials interrogating their role in KRAS mutant pancreas and lung cancer were subsequently considered a significant failure [38-41]. Further analysis following the clinical abandonment of FTIs offered more hope, emphasising the importance of considering RAS mutant isoforms when designing and delivering clinical trials: farnesylation was concluded to be of particular relevance to HRAS-driven cancer (with alternative mechanisms of membrane association/activation employed in the context of NRAS and KRAS mutations), and application of FTIs to HRAS mutant cell lines has subsequently proven effective [42].

More recently, the combination of docetaxel and the MEK inhibitor selumetinib in the phase II setting appeared to offer an incremental survival advantage in pretreated metastatic KRAS mutant
NSCLC, but the subsequent phase III SELECT-1 trial proved negative [15,43]. A phase II trial examining trametinib, another MEK inhibitor, was also shown to offer no discernible survival advantage compared to second line docetaxel in KRAS mutant disease [44].

One common feature of these studies which could help explain their lack of success was that contemporaneous biopsies were not mandated for KRAS testing – thus, prescient information on the background genetic context of individual tumours was unobtainable in the vast majority of patients. A new generation of NMR-developed small molecules which interact with the RAS-binding domains (RBDs) of proteins immediately downstream to KRAS now offer a potential route to success in this area, with one compound rigosertib undergoing clinical trial evaluation in haematological malignancies having demonstrated activity against a number of Ras effectors [45].

2. Synthetic lethality partners.

A number of synthetic lethality screens have been performed using KRAS mutant cancer cell lines, offering potential novel targets that include BCL-XL, TANK binding kinase-1 and CDK4 [46-48]. Many of these targets have associated direct small molecule inhibitors under preclinical assessment, and inhibitors of CDK4 are currently being tested in the clinic for treatment of 2nd to 3rd line NSCLC patients with KRAS mutant disease [49]. However the example of a previously identified synthetic lethality partner, STK33, suggested there are reasons to be cautious with this approach: following its identification using an RNA interference-based screen in KRAS mutant cell lines, a kinase inhibitor of STK33 proved ineffective [50]. A number of legitimate reasons have been offered to explain why there has been no breakthrough with synthetic lethality screens of RAS mutant cancer that match the success of PARP inhibition in BRCA mutant disease, including inconsistency of methodology, technology and cell line choice [33].
Why is there new optimism for KRAS mutation targeting?

Other than the encouraging progress seen with RBD inhibition described above, a number of direct inhibitors of mutant KRAS are now in early to late stages of preclinical development, with some expected to reach preclinical and clinical testing shortly (Table 1). Many of these inhibitors were designed with an appreciation that the crystal/NMR structures for each individual KRAS mutant subtype would be vital for progress.

The most advanced lead compound reported so far targets the most common G12C smoking-related subtype based on its crystal structure, using allosteric inhibition of a cysteine residue to prevent nucleoside exchange and regulation of G12C, thus holding it in the inactive GDP-bound state [51]. Second and third-generation iterations of this compound have demonstrated increasing biological activity measured by their ability to inhibit KRAS signalling [52,53]. Given its key role in cell homeostasis, avoiding off-target effects on wild-type KRAS will be a particular concern going forward, with not all compounds specifically targeted to its mutant subtypes and the Ras superfamily in general consisting of a network of 150 related proteins. However, the example of synthetic lethality screens, where no putative co-target could offer as potent an effect in KRAS-mutant cell lines as targeting KRAS itself with RNAi, suggests there is good reason to pursue this approach vigorously [33].

Other promising advances include pan-Ras compounds (which bind at least two sites on the Ras protein and have so far reported activity in the low micromolar range) and the identification of intracellular antibodies which can target oncogenic KRAS (Table 1) [54,55]. Although perhaps at an earlier stage of development compared to some other agents, the latter of these two prospects offers particular cause for excitement given it could broadly represent a new class of antibody which homes in on a tumour and internalises through receptor-mediated endocytosis.
Applying the same principals to what was observed 5-10 years ago with BRAF inhibitors in BRAF mutant cancer, it is expected that the ultimate success of these drugs will be contingent on their stratification to KRAS mutational subtypes such as G12C. In general, the increasing pursuit of direct KRAS inhibitors by both biotechs and academic institutions offer reasons to be hopeful, and the National Cancer Institute RAS initiative is another development which can support this programme of research in a number of different contexts.
Conclusions

Whether through an increased understanding of its genetic detail and molecular structure, or an improved practicality of identifying genetic subtypes with consistency in a large number of patients, the emergence of KRAS as a biomarker and target for biological agents should offer one of the next major breakthroughs in genetically-targeted treatment of cancer. However this will only represent the beginning of the process given a number of further complexities to delineate in the years ahead, including the potentiation of KRAS mutant-mediated malignant transformation by smoke-induced epigenetic changes, the role of KRAS in modulating the host immune response, and developing stories of resistance involving KRAS alteration or targeting [56-58]. Given its central role in mediating lung cancer, the world’s biggest cancer killer, as well as a number of other difficult-to-treat cancers, such a development could have an impact beyond anything seen before in molecular therapeutics.

2,663 words
CONFLICT OF INTEREST STATEMENT The authors have declared no conflicts of interest.
REFERENCES


## TABLES

<table>
<thead>
<tr>
<th>Name</th>
<th>Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lito et al, Science 2016 [53]</td>
</tr>
<tr>
<td>3144</td>
<td>Pan-Ras inhibition</td>
<td>Welsch et al, Cell 2017 [55]</td>
</tr>
<tr>
<td>RT11</td>
<td>Intracellular antibody</td>
<td>Shin et al, Nature Comm 2016 [54]</td>
</tr>
<tr>
<td><strong>DARPin</strong></td>
<td>Antibody mimetic inhibiting wild-type Ras nucleotide exchange</td>
<td>Guillard et al, Nat Commun 2017 [59]</td>
</tr>
<tr>
<td>SAH-SOS1A, SAH-SOS1B</td>
<td>Peptides to disrupt wild-type KRAS/SOS1 interaction</td>
<td>Leshchiner et al, PNAS 2015 [60]</td>
</tr>
<tr>
<td>SML-8-73-1, SML-10-70-1</td>
<td>GDP analogue that inhibits G12C</td>
<td>Lim et al, Angew Chem Int Ed Engl. 2014 [61]</td>
</tr>
<tr>
<td>Kobe 0065 and Kobe 2602</td>
<td>Small molecule inhibition of HRas-GTP-c-Raf-1 binding</td>
<td>Shima et al, PNAS 2013 [62]</td>
</tr>
<tr>
<td><strong>Andrographis paniculata (AGP) and its derivatives</strong></td>
<td>Inhibition of GTP-GDP exchange in KRAS wild-type and G12V</td>
<td>Hocker et al, PNAS 2013 [63]</td>
</tr>
<tr>
<td>Ras-DCAI</td>
<td>Small molecule to interfere with wild-type Ras-SOS interaction</td>
<td>Maurer et al, PNAS 2012 [64]</td>
</tr>
<tr>
<td>Not given</td>
<td>Small molecule to interfere with G12D Ras-SOS interaction</td>
<td>Sun et al, Angew Chem Int Ed Engl. 2012 [65]</td>
</tr>
<tr>
<td>F929, N944</td>
<td>Mimics protein-protein interaction to interfere with wild-type Ras-SOS interaction</td>
<td>Patgiri et al, Nature Chem Biol 2011 [66]</td>
</tr>
<tr>
<td>SCH 54292</td>
<td>Nucleotide exchange inhibiton of wild-type protein</td>
<td>Taveras et al, Bioorg Med Chem 1997 [67]</td>
</tr>
</tbody>
</table>

*Table 1.* Reported compounds in development that have directly targeted RAS.
**FIGURE LEGENDS**

**Figure 1.** *KRAS* mutational subtypes of lung adenocarcinomas analysed in phase I of the Cancer Research UK stratified medicine programme. NOS = mutation of KRAS codon 12 and/or 13 not otherwise specified.