

Review

KRAS-mutant non-small cell lung cancer: Converging small molecules and immune checkpoint inhibition



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ARTICLE INFO

Article history:

Received 19 December 2018
 Received in revised form 19 February 2019
 Accepted 21 February 2019
 Available online 7 March 2019

ABSTRACT

KRAS is the most frequent oncogene in non-small cell lung cancer (NSCLC), a molecular subset characterized by historical disappointments in targeted treatment approaches such as farnesyl transferase inhibition, downstream MEK inhibition, and synthetic lethality screens. Unlike other important mutational subtypes of NSCLC, preclinical work supports the hypothesis that *KRAS* mutations may be vulnerable to immunotherapy approaches, an efficacy associated in particular with TP53 co-mutation. In this review we detail reasons for previous failures in *KRAS*-mutant NSCLC, evidence to suggest that *KRAS* mutation is a genetic marker of benefit from immune checkpoint inhibition, and emerging direct inhibitors of K-Ras which will soon be combined with immunotherapy during clinical development. With signs of real progress in this subgroup of unmet need, we anticipate that *KRAS* mutant NSCLC will be the most important molecular subset of cancer to evaluate the combination of small molecules and immune checkpoint inhibitors (CPI).

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1. Introduction

Over the past 15 years the treatment of NSCLC has changed dramatically with the development of molecular profiling, targeted therapeutic agents, and precision medicine [1]. In NSCLC somatic mutations in *EGFR* and rearrangements in *ALK*, *ROS*, and *RET* have been validated as strong predictive biomarkers and attractive drug targets [2–7]. Historically Ras has been described as an “undruggable” target [8], and despite more

than three decades of effort, no effective anti-Ras inhibitors are currently used in routine clinical practice.

The *RAS* family encode small enzymes that hydrolyse guanosine triphosphate (GTPase), linking upstream cell surface receptors such as *EGFR*, *FGFR*, and *ERBB2–4* to downstream proliferation and survival pathways such as *RAF–MEK–ERK*, *PI3K–AKT–mTOR*, and *RALGDS–RA* [9]. It is the most frequent oncogene in cancer with mutations of *KRAS*, *NRAS*, and *HRAS* occurring in 30% of cases. *KRAS* is the isoform most commonly mutated in 86% of *RASm* cancer cases, followed by *NRAS* 11% and *HRAS* 3% (Fig. 1) [8]. The most frequent rates of *RAS* modification are found in lung, pancreatic, and colorectal adenocarcinoma: *KRAS* being most common in lung, pancreatic, and colon cancer. *NRAS* in melanoma, and *HRAS* in bladder cancer [10]. *KRAS* mutations occur in 20–40% of lung adenocarcinomas, a prevalence that is higher in

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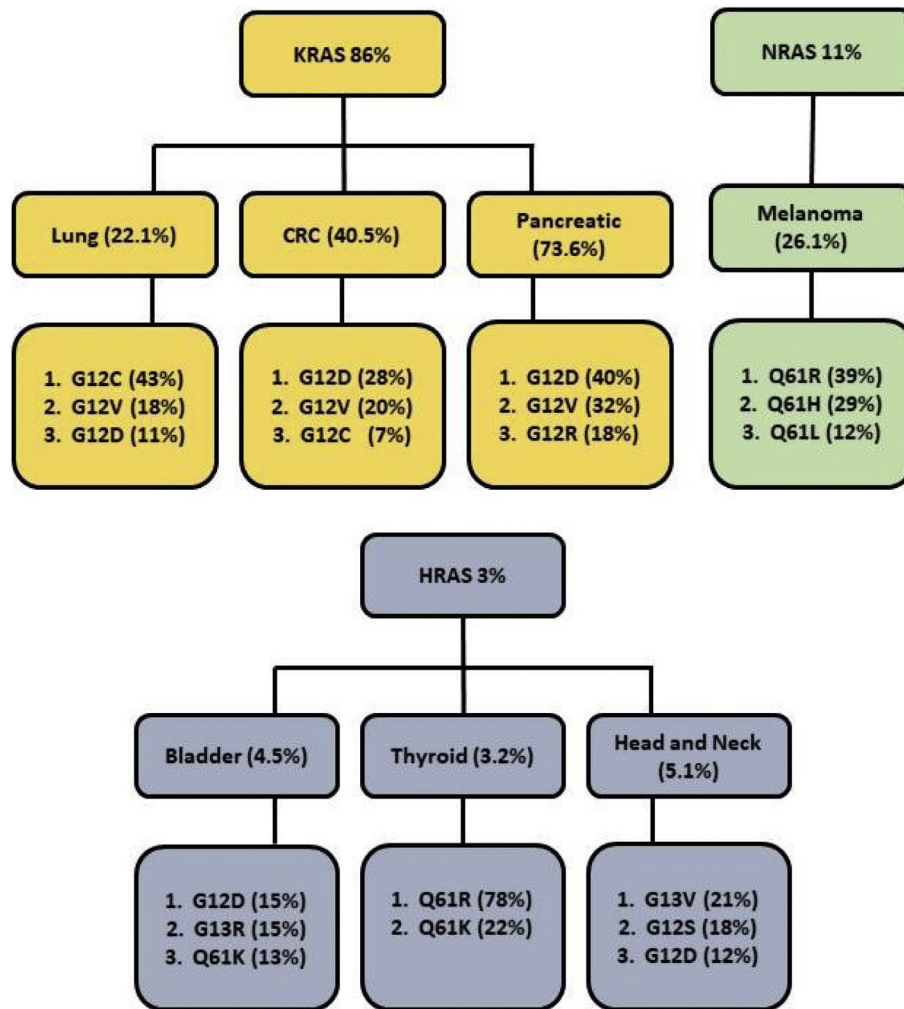


Fig. 1. Frequency of RAS mutation subtypes: KRAS, NRAS, HRAS.

Western vs Asian populations (26% vs. 11%) and smokers vs non-smokers (30% vs. 10%) [11]. The most frequent mutations occur in codons 12 and 13, with the most common subtypes including G12C, G12V, and G12D (Fig. 1). Common KRAS co-mutational partners have been identified in NSCLC, most frequently TP53 (40%), STK11/LKB1 (32%) and CDKN2A (19.8%). These subgroups tend to be mutually exclusive and appear to have no contextual preference between KRASm alleles [12–15].

Frequency of most common RAS mutations, followed by overall prevalence of mutations and their common alleles in RASm-associated cancers.

2. Failures in KRAS mutant targeting

The unprecedented challenge of effective KRAS targeting is evidenced by the disappointing results of three main treatment approaches to date. First, failed trials of farnesyl transferase inhibitors were abandoned following the discovery that K-Ras and N-Ras could employ geranyl-geranylation as an alternative mechanism to farnesylation for activation of oncogenic K-Ras [16–18]. Second, downstream inhibition of MEK using selumetinib in combination with docetaxel, recently investigated in the phase III Select-1 trial, failed to show significant improvements of survival or response [19] (PFS 3.9 vs 2.8 months; HR 0.93; 95% CI 0.77–1.12; $p = 0.44$) (OS 8.7 vs 7.9 months HR 1.05; 95% CI 0.85–1.30; $p = 0.64$), findings that were consistent with a large KRASm-selected phase II trial examining second line trametinib vs. docetaxel (PFS 12 vs 11 weeks; HR 1.14; 95% CI 0.75–1.75; $p = 0.5197$) [20]; further detail on the translational

output of both studies is eagerly anticipated, and it will be interesting to examine whether subdivision according to factors such as KRASm alleles or co-mutational partners could offer differential efficacy signals. This possibility has been supported by recent preclinical work identifying that KRAS allelic imbalance is frequent (55% of a 1100 cohort) and has a bearing on MEK dependency [21]. LOH and disruption of K-Ras dimerization were also characterized as potential predictors of MEK inhibitor benefit in KRASm tumours [22].

Finally, a number of synthetic lethality screens have been performed using KRASm NSCLC identifying targets including BCL-XL, TANK binding kinase-1, and CDK4 [23–37]. One main hit from these studies is CDK4, for which abemaciclib has been employed as a selective small molecule inhibitor in phase I-III clinical trials of KRASm disease [38]. Results so far have not been encouraging with this approach, although we await more detail from both the phase III JUNIPER study [39] and forthcoming reports from the Cancer Research UK MATRIX trial assessing an alternative CDK4 inhibitor, palbociclib [40,41].

3. Is RASm predictive of immune checkpoint inhibitor response in NSCLC?

As CPIs are now used as standard therapy in a majority of NSCLC patients, identifying molecular subtypes that provide predictive value will be critical for selection of appropriate patients. The benefit of CPIs were originally demonstrated in second line NSCLC, where nivolumab was first evaluated in Checkmate-017 [42] and Checkmate-057 [43]. These pioneering results were quickly followed by confirmation that

pembrolizumab and atezolizumab also offered good options for second line treatment of NSCLC, a benefit that was agnostic of PD-L1 status in some cases [44,45]. However it is in the stage III and first line stage IV setting where CPIs have made their most striking breakthroughs to date, including confirmation of clear benefits for pembrolizumab monotherapy in patients with PD-L1 expression by immunohistochemistry >50% (pembrolizumab monotherapy), and chemotherapy/pembrolizumab combination in all other patients with stage IV disease [46–48]. The future of CPIs therefore knows no limits in NSCLC at present, with recent data suggesting it may eventually be employed in the neoadjuvant setting – a question which a number of phase III trials are now pursuing further [49]. (NCT02259621).

The key limitation of the above advances has been the identification of a biomarker that can sensitively and specifically predict treatment response. PD-L1 immunohistochemistry and assessment of tumour mutation burden (TMB) currently represent the most clinically tested predictive biomarkers, although their limitations have been well characterized [50–53]. Better reporting of RASm potentially has predictive importance for CPI efficacy in NSCLC, although studies have so far not uniformly offered positive results. It however remains compelling to hypothesise that an increased NSCLC mutational burden (and likely neoantigen increase) via smoking could be represented by RASm as a common marker for treatment efficacy. Mechanistic insight to support this hypothesis has been offered by the Crick Institute, who have shown that oncogenic K-Ras signalling can stabilise PD-L1 mRNA via post-transcriptional changes to the AU-rich element-binding protein, TTP [54].

Individual randomised controlled trials have not been designed or powered to examine treatment difference between molecular subgroups of NSCLC, although two meta-analyses have reviewed this possibility. The first identified three randomised phase II or III clinical trials examining OS in KRASm NSCLC [43,44,55] (Table 1), concluding that CPIs as second or third line therapy in KRASm NSCLC improve OS compared to standard chemotherapy [56]. There was no significant OS benefit between immunotherapy and chemotherapy in KRAS WT NSCLC, leading the authors to hypothesise that KRASm status could be used as predictive biomarker when selecting patients for immune checkpoint inhibitors. The second meta-analysis examined the same three clinical trials, citing a pooled HR of 0.65 (95% CI 0.44–0.97, p = 0.03) for the KRASm subgroup (148 patients, 28.5%) [57]. As there was no significant treatment interaction for KRAS mutation in this study (KRASm HR 0.86 vs. KRAS wild type HR, 0.65; p = 0.24), Lee and colleagues concluded that there is not enough evidence to recommend KRASm alone as a predictive biomarker for CPIs. They did however conclude that KRASm was associated with increases in tumour infiltrating lymphocytes, PD-L1 expression and TMB.

Using real-world data, two recent studies have given further insight toward the predictive potential of KRASm. First, Passiglia and colleagues [58] evaluated the efficacy of nivolumab in 206 pretreated KRASm NSCLC patients, demonstrating that KRASm status did not confer significant differences in ORR, PFS or OS. The only significant change noted

between KRASm vs. KRAS WT cohorts was at 3-month PFS, although co-mutations including TP53 and LKB1 were not evaluated in this cohort and may have had an influence. These results were consistent with a second study examining 162 KRASm patients treated with CPI, which also detailed that KRASm alleles appear to confer no further influence on CPI benefit [59]. This article analysed PD-L1 status, demonstrating that mean PD-L1 expression in KRASm is 22.13% [95% CI 14.66–29.6] vs. 15.65% for KRAS WT disease. [95% CI 6.11–26.83]. It also suggested that PD-L1 positivity was associated with G12D, G12V or G13C KRASm cancers.

Taken together, it remains clinically unproven that the categorical identification of KRASm or not will suffice to predict CPI response, although more data will undoubtedly emerge in this space given the pre-clinical biology to support this hypothesis. In contrast to other genetic subgroups of NSCLC (such as EGFR-mutation or ALK-rearrangement) that are considered from preclinical and clinical trial work to be ‘immune-cold’, the path forward for KRASm patients may soon be dominated by combination trials involving CPIs and small molecules.

4. Are RASm subgroups the key?

Molecular and environmental diversity of KRASm subgroups in NSCLC offers an attractive biological explanation for the above disparity in results [60]. Skoulidis and colleagues [12] examined the diverse heterogeneity of KRASm NSCLC analysing data from early stage and chemo refractory disease.

In this article, which defined three KRASm subsets according to presence of co-mutations including STK11/LKB1 (‘KL’), TP53 (‘KP’), and CDKN2A/B inactivation (‘KC’), it was concluded that these subgroups drive biological diversity which would require fundamentally different approaches to targeted treatment. In particular the KL subgroup, was associated with an inert tumour immune microenvironment and poor clinical response to immune checkpoint blockade. Although the mechanism of this phenotype was unclear, it may be linked to a lower level of somatic mutations with reduced expression of immune checkpoints. LKB1 has also generally been linked to a recalcitrant phenotype in KRASm cancer via its effects on oxidative metabolism and the epithelial mesenchymal transition [61,62]. In contrast to KL, KP tumours were characterized by an inflammatory response, immune-editing and expression of co-stimulatory and co-inhibitor molecules including PD-L1, suggesting that this subtype may be particularly susceptible to immune checkpoint inhibition. All of these results were recently updated with an assessment of CPI efficacy in the 3 identified co mutated groups, demonstrating a significant difference in ORR between subgroups in the SU2C cohort: 7.4% KL vs. 35.7% KP vs. 28.6% K-only (p < 0.001) and in the CM-057 cohort ORR: 0% KL vs. 57.1% KP vs. 18.2% K-only (p = 0.047) [13]. PD-L1 expression varied significantly across subgroups, with KL tumours least likely to be PD-L1 positive. KP tumours had the highest rates of PD-L1 positivity at 56.3% vs. 32.3% in KRAS WT, while mean TMBs across KL and KP alterations were comparable ranging from 8.1 to 11.7 mutations/Mb. The association of KL co-mutation and

Table 1
KRASm NSCLC response to immunotherapy in studies to date.

Study name, year	Phase	Setting	Arms	No. KRASm patients	Progress
CheckMate 057, 2015 [43]	III	2nd line	Nivolumab 3 mg/kg 2 weeks vs. Docetaxel	62	Median OS 12.2 vs. 9.4 months OS HR 0.52 (95% CI 0.29–0.95)
POPLAR, 2016 [55]	II	2nd /3rd line	Atezolizumab 1200 mg 3 weeks vs. Docetaxel	27	Median OS 12.6 vs. 9.7 months OS HR 0.94 (95% CI 0.36–2.45)
OAK, 2017 [44]	III	2nd/3rd line	Atezolizumab 1200 mg weeks vs. docetaxel	59	Median OS 13.8 vs 9.6 months OS HR 0.71 (95% CI 0.38–1.34)
NCT03299088	Ib	2nd line	Pembrolizumab +trametinib	Estimated 42	Recruiting
KEYNOTE 001, (subgroups analysed by Dong et al., 2017) [63]	Post hoc analysis of phase I	1st line	Pembrolizumab	8	Median PFS KRASm 14.7 vs. 14.5 TP53m vs. 3.5 KRAS wt

low PD-L1 expression was consistent across the SU2C and CM-057 cohorts, 13.6% and 11.1% respectively. In over 900 KRASm patients, *STK11/LKB1* was the only marker significantly associated with PD-L1 negativity in intermediate to high TMB disease. The negative impact of this subgroup also extended to PD-L1 positive NSCLC. Authors concluded that *STK11/LKB1* alterations play a major role in primary resistance to CPI blockade in NSCLC.

The narrative of KRASm co-mutations is supported by results from Dong and colleagues who showed that the *TP53/KRAS* co mutation resulted in increased expression of PD-L1 and a high proportion of PD-L1 +/CD8A+ [63]. This study hypothesised that patients with both mutations had increased sensitivity to PD-1 blockade, re-analysing publicly available data from keynote 001 which included 34 advanced stage NSCLC patients prescribed pembrolizumab from 2012 to 2013 using the NCT01295827 protocol (Table 1). *TP53* or *KRAS* mutant patients demonstrated a significantly prolonged PFS vs. WT patients who received pembrolizumab (median PFS *TP53m* vs. *KRASm* vs. WT: 14.5 vs. 14.7 vs. 3.5 months $p = 0.012$). Patients with co-occurring *TP53* and *KRAS* mutations showed remarkable clinical benefit to PD-1 inhibitors.

The possibility of prospective data in this space may not be forthcoming and, with cumulative translational evaluation from existing and future clinical trials, we may soon be forced to conclude that the KRASm *LKB1*-deficient group (10% of NSCLC patients) is a recalcitrant subset which urgently requires drug combinations to sensitise CPI response. Another key clinical/translational question will be to examine the differential CPI responses from *KRAS* alleles, although data so far has suggested that they have no clear association with *TP53/LKB1* subgroups [12,64]. Finally, we should consider whether simple gene tests such as *TP53* and/or *LKB1* can predict CPI response more accurately than the current standards of PDL1 immunohistochemistry and estimation of TMB [50,52]. One potential advantage of this would be that CPI prediction could be more conveniently rolled out to include circulating tumour DNA, reducing our current reliance on tumour tissue.

5. Looking ahead

In forging a path forward for KRASm NSCLC, future approaches may involve CPI combination with developing small molecule inhibitors. Despite previous failures with small molecule therapy in KRASm disease, recent developments have highlighted reasons to be optimistic including a number of direct inhibitors of oncogenic K-Ras in pre-clinical development. The most advanced of these compounds target the *G12C* subtype typical to NSCLC, preventing nucleotide exchange and maintaining K-Ras in an inactive GDP bound state [65–67]. Two oral small molecules, AMG 510 (NCT03600883) and MRTX849 (NCT03785249), are now being evaluated in phase I clinical trials. Other pre-clinical developments include pan-RAS compounds, SHP2 inhibitors, and intracellular antibodies that target oncogenic K-Ras [68–71]. Downstream in the Ras pathway, promising preclinical work has suggested selective RAF dimer inhibitors (e.g. RAF709) can also induce responses in RASm tumours [72]. In a manner analogous to 3rd generation ALK or EGFR inhibitors in lung cancer, the hope is that new drugs for ‘old’ targets will offer significant improvements compared to their predecessors. All of them will hopefully be introduced to a clinical landscape that includes KRASm-directed studies such as NCT03299088, aiming to evaluate the combination of CPI and MEK inhibitor in *KRAS* mutant NSCLC, and the Cancer Research UK Matrix study [73] NCT02664935, which evaluates CDK4/6 inhibition in a more genetically selected KRASm cohort than that evaluated in the JUNIPER study [39]. Vital insight will be obtained from such studies, hopefully informing a further wave of trial development on top of iterative translational research to examine causes of response/resistance to CPI combinations. Clinical development and progress will be dependent on correct assessment of patients according to their various mutational *KRAS* subtypes, as well as toxicity between

CPIs and small molecules. Occasional reports of severe side-effects so far offer reasons to be cautious [74].

6. Conclusions and outstanding questions

The recent characterisation of a direct mechanistic link between oncogenic *KRAS* and stabilisation of PD-L1 mRNA has offered a timely reminder that preclinical/clinical research assessing tumour genetics and the tumour micro-environment must be considered in the same space. Clinical data is emerging to suggest that patients with KRASm NSCLC perform better with CPIs, particularly when their cancers have wild-type *LKB1*. Whilst a new raft of clinical trials assessing CPI/small molecule combinations is expected in KRASm disease, more work should follow to address whether simple assessment of *KRAS* and its key co-mutations can offer predictive insight for treatment responses. Reports of significant responses to T-cell adoptive therapy in KRASm cancer will also merit further interrogation [75].

7. Search strategy and selection criteria

Data for this review was included by searches of MEDLINE and PUBMED, with reference from relevant articles including “KRAS”, “NSCLC”, “immunotherapy” and “Targeted therapy”. Only articles and abstracts were included from 1995 to 2019 published in English Language from peer reviewed sources.

Disclosure

Dr. Blackhall reports grants from AstraZeneca, grants from Novartis, grants from Pfizer, grants from Amgen, grants from BMS, other from Regeneron, other from Medivation, other from AbbVie, other from Takeda, other from Roche, other from Ibsen, non-financial support from CellMedica, non-financial support from MSD, other from Boehringer Ingelheim, outside the submitted work.

Dr. Lindsay has received institutional as a site PI for a Roche-sponsored study.

Author contributions

Construction of review was performed by HA and CL. Review was performed by CL and FB. All authors read and approved final manuscript.

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