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# Expanding *KRAS* Gene Testing: We're Ready and Here's Why

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## Abstract

**Purpose:** The aim of this review was to address the barriers limiting access to the use of molecular diagnostics, specifically *KRAS* testing for cancers with potential to benefit from targeted drugs in Brazil. A panel reviewed examples from current state and potential future uses of *KRAS* testing in cancer diagnosis and treatment designation.

**Design:** A selected panel of Brazilian experts in fields related to *KRAS* testing were provided with a series of relevant questions to address prior to the multi-day conference. Within this meeting, each narrative was discussed and edited by the entire group, through several drafts and rounds of discussion until a consensus was achieved.

**Results:** The authors propose specific and actionable recommendations for expanding access to *KRAS* testing use in cancer care in Brazil and in other countries, in a similar situation. In creating these recommendations, the authors strived to address all barriers and impediments mentioned previously within this review.

**Conclusion:** Given the current benefits and likely future applications, there is a great need to expand molecular testing and *KRAS* testing in Brazil, and adapting the current framework is essential to accomplishing this goal. Regulatory actions and increased knowledge and awareness to expand access to this technology have the potential to improve cancer patient care across the country. Therefore, the recommendations in this review can serve as an outline for technology adoption in Brazil and other countries with similar challenges in optimizing cancer care.

**Keywords:** KRAS • Molecular testing • Cancer • Brazil • Molecular tool • Precision

## Introduction

Advancements in molecular testing enable physicians to use precision medicine in treatment of cancer patients harbouring actionable mutation. *KRAS* is one of the most commonly mutated genes in cancer, along with *p53, PIK3CA, ATM, PTEN*, *APC*, *BRAF* and several others [1]. *KRAS* mutations are present in three of the most highly prevalent and deadly cancers: lung, colon and pancreas. Recent advances in evidence-based therapeutics presented promising clinical benefits for patients harbouring such tumours. Historically, mutated KRAS has been un-druggable despite the progress in targeted therapies. Recently, however, several KRAS-targeted drugs are the subject of ongoing clinical trials. Thus, it is critical that Brazil sets the stage for adoption and implementation of precision medicine and their associated tests as a model for other countries in similar situations.

## Literature Review

To address the above issues, the Americas Health Foundation (AHF) conducted a literature review to identify scientists and clinicians from Brazil who have published in molecular testing and, specifically, *KRAS* testing. Pub Med and Embase were used to identify clinicians and scientists with an academic or hospital affiliation, and who had published in molecular oncology since 2012. Augmenting this search, AHF contacted several individuals in various countries in Latin America to derive a list of individuals suitable for the project. As a result of this effort, AHF convened a six-member panel of clinical and scientific experts from Brazil, representing the disciplines of precision medicine, oncology, pathology, genetics and applied genomics. Great attention was paid to ensure a diverse group representing various disciplines related to *KRAS* testing.

### Search Strategy and Selection Criteria

Papers useful for the consensus discussion and the references cited in this paper were identified through searches of Pub Med and Embase with the search terms "molecular testing", "targeted therapy", "*KRAS* testing" and "companion diagnostics" from 2012 until 2019. Articles were also identified through the bibliographies of the papers identified in the search as well as from searches of the authors' own files. Particular attention was paid to papers that reviewed or summarized the topic in question, or that were related to activities in Latin America, especially Brazil. The final reference list was generated on the basis of the relevance to the broad scope of this consensus document.

To better focus on the discussion, AHF staff independently developed specific questions for the Panel to address. The questions were selected to address the salient issues on the subject. On the first day of the multi-day meeting of the Panel, each question was discussed at length and an outline for the answer to each question was established. Subsequently, a written response to each question was initially drafted by individual Panel members and each narrative was edited by the entire group through numerous drafts and rounds of discussion until complete consensus was obtained. Subsequent

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to the meeting, the Panel was asked to review the document and to again acknowledge that they were in full-agreement.

## Incidence and burden of associated KRAS cancer in Brazil

Cancer is a major concern for modern society. In 2018, about 43.8 million people were living with cancer worldwide, with 18.1 million new cases and 9.6 million cancer deaths [2]. In Latin America, the burden of cancer is significant, due to delays in diagnosis, with frequent advanced diseases at presentation and limited access to innovative diagnostic and treatment modalities [3].

In Brazil, 625,370 new cases of cancer were estimated for 2020. The incidence rate for men and women is comparable to those seen in more development countries. The most frequent cancer types in men are prostate (29.9%), lung (9.1%), colorectal (7.9%) stomach (5.9%) and oral cavity (5%). In woman, the main tumor types are breast (29.7%), colorectal (9.2%), cervix (7.4%), lung (5.6%) and thyroid (5.4%) [4].

Cancer is already the first cause of death in 10% of the municipalities in Brazil (516 cities) [5]. In 2019, cancer was responsible for 232,030 deaths (121,686 in men; 110,344 in women), representing 16.6% of total deaths in the country. Breast cancer had the highest mortality rate among women in 2019, followed by lung, colorectal, cervical and pancreatic. Among men, lung is followed by prostate, colorectal, stomach and esophageal cancers [6].

*KRAS* is one of the most common oncogenes found in human cancer [7]. *KRAS, HRAS* and *NRAS* are part of the RAS family of genes. All of them code for very similar proteins that control cell growth and differentiation. Mutated *ras* proteins become constitutively activated and so function as a proliferation stimuli.

*KRAS* plays an important role in the epidermal growth factor receptor (EGFR) signaling pathway that controls cell growth and proliferation (Figure 1). EGFR pathway signaling is triggered by an alteration in RAS proteins, which is due to activating mutations in *KRAS* gene. This pathway signaling a common event in several types of cancer and is regarded as a prominent step in tumorigenesis. The most commonly mutated codons in *KRAS* are 12, 13, and 61, which create drug resistance to EGFR tyrosine kinase inhibitors (EGFR-TKIs). Several studies suggest that *KRAS* mutations should be known prior to using EGFR-TKI therapies. Although tumor tissue is the standard source for *KRAS* mutation detection, obtaining tissue samples is invasive, and may be associated with additional cost, which may not always be feasible. Considering the advantages and disadvantages of tissue testing, liquid biopsy offers an opportunity to access tumor tissue not only for diagnosis but also for serial sampling and follow up [8,9].

Approximately 30% of human cancers involve genes of the RAS family, and among those, the great majority (approximately 85%) are due to *KRAS* mutation [10]. Among the tumors with the highest frequency of *KRAS* mutations are lung, colorectal, and pancreatic cancer affecting an estimated 5% of the cancer population in Brazil [4].

Lung cancer is the most frequently diagnosed cancer worldwide and the leading cause of death [11]. In Brazil, as previously stated, it is the second most frequent malignant tumor type among men and the fourth in women, with an estimation of more than 30,000 new cases per year [4]. *KRAS* mutations are seen in approximately 30% of all lung cancers. Non-small cell lung cancer (NSCLC) accounts for approximately 85% of these tumors. Within NSCLC, *KRAS* mutations have been reported in 25–34% of adenocarcinomas and in 2-6% of squamous cell carcinomas [12-14]. In adenocarcinomas, the most common codon 12 substitutions are G12C and G12V, both associated with smoking history, whereas the G12D is more frequent in never smokers [15]. In some series, KRAS mutations have been related to worse treatment outcomes and considered a in depend variable, with deleterious prognostic value [16].

Colorectal cancer (CRC) is the third most frequent cancer in the world and the second cause of mortality, after lung cancer [2]. In Brazil, CRC is the third most frequent cancer in men and the second in women. *KRAS* mutation is seen in approximately 40% of CRC [17]. The use of anti-EGFR targeted therapies is a treatment option for metastatic CRC patients whose tumors are wild type *KRAS* (wt*KRAS*) [18].

In CRC, like in most tumors with *KRAS* mutation, codon 12 is the most frequently affected with p.G12D (c.35G>A) being the most common change. Alterations in other codons like 13, 59, 61, 117, and 146, although less frequent, might also be seen [17]. Mutation in *KRAS* is a driver event in CRC and is very homogenous among primary and metastatic tissue samples [19].

CRC originating from the right or left side colon are distinct clinic pathological entities. Although *KRAS* mutations are more frequent on the right-side, it is only associated with poorer prognosis when present on the left-side [20]. KRAS mutations in colon cancers, and in lung cancers, have been associated with poorer survival and increased tumor aggressiveness [21].

Pancreatic adenocarcinoma, in its turn, is one of the most lethal cancers and ranks among the ten most frequent cancers in the world. In Brazil, there were over 10,000 deaths in 2017 due to pancreatic cancer, which usually develops and grows clinically silent and frequently progresses to unrespectable disease, before becoming noticeable. The 5-year survival rate is less than 7% [22]. *KRAS* constitutive activation is a driver event in pancreatic neoplasia, being detected in the primary and metastatic scenario, and also in pre-neoplastic lesions [22,23]. The clinical utility regarding *KRAS* mutation detection in pancreatic tumors is still strengthening. Pancreatic ductal adenocarcinoma displays 100% of *KRAS* mutation frequency. Therefore, this cancer could be a model to determine if these mutations respond differentially to drugs targeting either KRAS itself or its downstream effectors, such as PI3K, AKT, MEK an ERK [23].

Specifically in Brazil, the frequency of *KRAS* in tumors has been reported by several studies, as seen in Table 1. In NSCLC, the frequency ranges from 8% to 30.2%. In CRC, it ranges from 18.3% to 58.9%. In pancreatic adenocarcinoma, it ranges from 60% to 78.8%. These variations may be because of the differences in genetic ancestry of the Brazilian population and the technology used. Most studies have been conducted in the South and Southeast regions, which stresses the inequities in resource distribution throughout the country, including lack of investment, competing priorities, shortage of healthcare personnel, among others [24-47].

## Brazilian healthcare system

Brazil is the largest country in Latin America, with a population of approximately 216 million inhabitants and a *per capita* gross domestic product (GDP) of $15,600 USD, according to 2017 data [47,48]. The 1988 Constitution established that all Brazilian citizens have the right to comprehensive health care, through *Sistema Único de Saúde* (SUS), which is unique in the continent, funded by taxes and insurance payments [49]. A complimentary system, private healthcare, exists for those who can pay.

The country spends $1,318 USD *per capita* in healthcare, a little over 8% of its GDP, which is close to the regional average. However, SUS and

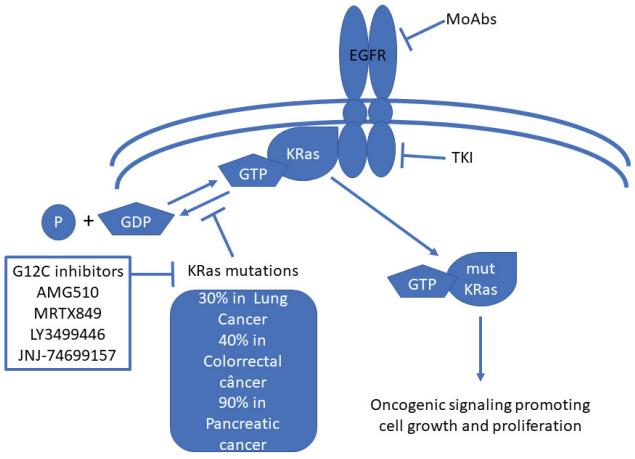


**Figure 1.** The *EGFR* pathway – *KRAS* is an important downstream effector of the *EGFR* pathway and several inhibitors targeting *KRAS G12C* are undergoing clinical testing.

**Table 1.** *KRAS* frequency in lung, colorectal, and pancreatic tumors in Brazil.

| Study | Year | Type of Tumor | Number of Cases | Technology Used (Codon Evaluated) | *KRAS* Mutation Frequency | *KRAS* G12C Frequency (From Total Number Of Cases Tested) | Ref |
|---|---|---|---|---|---|---|---|
| Mascarenhas E | 2021 | NSCLC | 513 | CGP (12,13) | 24.2% | 25.4% | 24 |
| Araujo LH | 2021 | NSCLC | 4686 | NGS | 21.4% | 7.4% | 25 |
| Freitas HC | 2020 | NSCLC | 495 | NGS (12,13, 61,117,146) | 26.9% | 9.5% | 26 |
| Andreis TF | 2019 | NSCLC | 619 | NGS (12,13,61) | 30.2% | 11.1% | 27 |
| Leal LF | 2019 | NSCLC | 444 | Sanger (12;13) | 20.4% | 7.2% | 28 |
| Almeida TA | 2019 | NSCLC | 121 | Pyrosequencing (12,13,61) | 21.5% | 6.6% | 29 |
| Gomes JR | 2017 | NSCLC | 290 | NA | 20.0% | NA | 30 |
| de Melo AC | 2015 | NSCLC | 125 | Sanger/ Pyrosequencing (12;13) | 26.4% | 12.8% | 31 |
| Carneiro JG | 2014 | NSCLC | 88 | Sanger (12, 13,61) | 8% | 3.4% | 32 |
| Bacchi CE | 2012 | NSCLC | 206 | Sanger/Real time (Taqman) | 14.6% | 7.3% | 33 |
| Araujo LH | 2021 | Colorectal | 4897 | Pyrosequencing (12,13, 61, 117,146) | 48.1 | 3.4 | 25 |
| Zanatto RM | 2020 | Colorectal | 69 | Direct sequencing and pyrosequencing (12,13) | 43.3 | | 34 |
| Pereira AAL | 2020 | Colorectal | 1635 | PCR (12,13, 59,61,117, 146) | 17% | NA | 35 |
| Silva ACB | 2019 | Colorectal | 307 | NA | 37.4% | NA | 36 |
| Miranda RR | 2019 | Colorectal | 47 | NGS (12,13, 61,117,146) | 34% | NA | 37 |
| dos Santos W | 2019 | Colorectal | 91 | NGS (12,13, 61,117,146) | 52.7% | 3.3% | 38 |
| Usón Jr PLS | 2018 | Colorectal | 151 | PCR (12,13, 146) | 58.9% | 11.3% | 39 |
| de Carvalho LEW | 2017 | Colorectal | 60 | Sanger (12,13) | 18.3% | NA | 40 |
| Macedo MP | 2016 | Colorectal | 102 | Pyrosequencing (12,13,61) | 40.1% | 2% | [41] |
| Ferreira EN | 2015 | Colorectal | 463 | NGS (12,13, 61,117,146) | 46% | NA | 42 |
| Macedo MP | 2015 | Colorectal | 422 | Pyrosequencing (codon 12 and 13) | 33.4% | <1% | 43 |
| Ferreira CG | 2014 | Colorectal | 8234 | Sanger (12,13) | 31.9% | 2.5% | 17 |
| Ribeiro KB | 2013 | Colorectal | 65 | NA | 49.2% | 6.2% | 44 |
| Kubrusly MS | 2002 | Pancreatic | 97 | PCR/RFLP (12) | 78.8% | NA | 45 |
| Amaral NS | 2018 | Pancreatic adenocarcinoma | 23 | Sanger | 60% | 0% | 46 |

private systems have sizeable inequity gaps where 80% of the population rely exclusively on SUS and spend less than half of the total healthcare budget [50].

The Brazilian Health Regulatory Agency (ANVISA) is in charge of the approval of registration of health technologies in the country. The incorporation of new technologies is subjected to the approval of different agencies in the public and private system. In SUS, the National Committee for Technology Incorporation (CONITEC) is the governmental agency responsible for assisting the Ministry of Health in incorporating or excluding health technologies [51].

In SUS, reimbursement for oncologic treatments is based on a set monetary amount for every clinical oncologic diagnosis (site and stage) regardless of the treatment selected. A very limited number of antineoplastic drugs are provided directly by the Ministry of Health [52]. As a result, SUS-accredited hospitals purchase and provide oncologic treatment but are restricted by the reimbursement budget defined by the central government [53].

A disconnection between drug and companion testing approval exists in the country, creating a contradictory scenario in clinical practice predominantly in SUS [54]. Thus, targeted drugs may be incorporated, but tests to support the use of these medications are not reimbursed, hence not broadly implemented.

In the private system, all patients have access to the procedures included in a list published and revised every two years by the National Healthcare Agency (ANS) [55]. A multi-stakeholders committee (COSAÚDE) evaluates and advises the private system regarding the incorporation of new technologies, but this evaluation is subject to further approval processes by ANS [56]. Insurance companies are free to extend their coverage beyond the procedures mentioned in the list, affecting annual premiums.

Even though technology to process molecular diagnostics is available in Brazil, ANS reimbursement for companion diagnostics is only ensured for on label treatments. Currently, *KRAS* testing is only covered for metastatic colorectal cancer when the patient is a candidate for anti-EGFR therapies.

## Molecular testing

Tissue samples remain the gold standard for analyzing the molecular landscape for solid tumors. However, there are many limitations in conventional sampling methods, such as complications associated with the procedures (reported as 1 in every 6 biopsies), difficulties in obtaining sufficient material of adequate quality for molecular testing (reported failure rates range from 10% to 30% of cases), limited representation of tumor heterogeneity, and difficulties in sequential tissue biopsy sampling (Figure 2) [57].

An alternative to this tissue-based approach is the use of liquid biopsy, which, in some studies, has shown high concordance between the results from the tissue and corresponding tumors when collected at the same time. These results have important implications for both molecular pathology and clinical oncology [58].

In oncology, liquid biopsy is a term that refers to tumor derived markers that can be detected in body fluids, such as blood or urine. Three components of liquid biopsy are the most used to investigate tumor-derived material: circulating tumor DNA (ctDNA), circulating tumor cells and subcellular particles or exosome [59]. In precision oncology, liquid biopsy mainly refers to the analysis of ctDNA that enables the screening of tumor specific markers, such as tumor somatic mutations [60].

Cell-free DNA (cfDNA), is released from both healthy and tumor dying cells into the circulation. Evidence shows that levels of cfDNA rises with stress, tissue injury or inflammation and that patient with cancer have more cfDNA than healthy people [61]. ctDNA normally represents a fraction of the cfDNA isolated from blood. Identifying specific tumor mutations that are being tested can be distinguished from normal DNA. A negative result does not rule out the presence of the mutation in the tumor, due to the low negative-prediction value of the method [60].

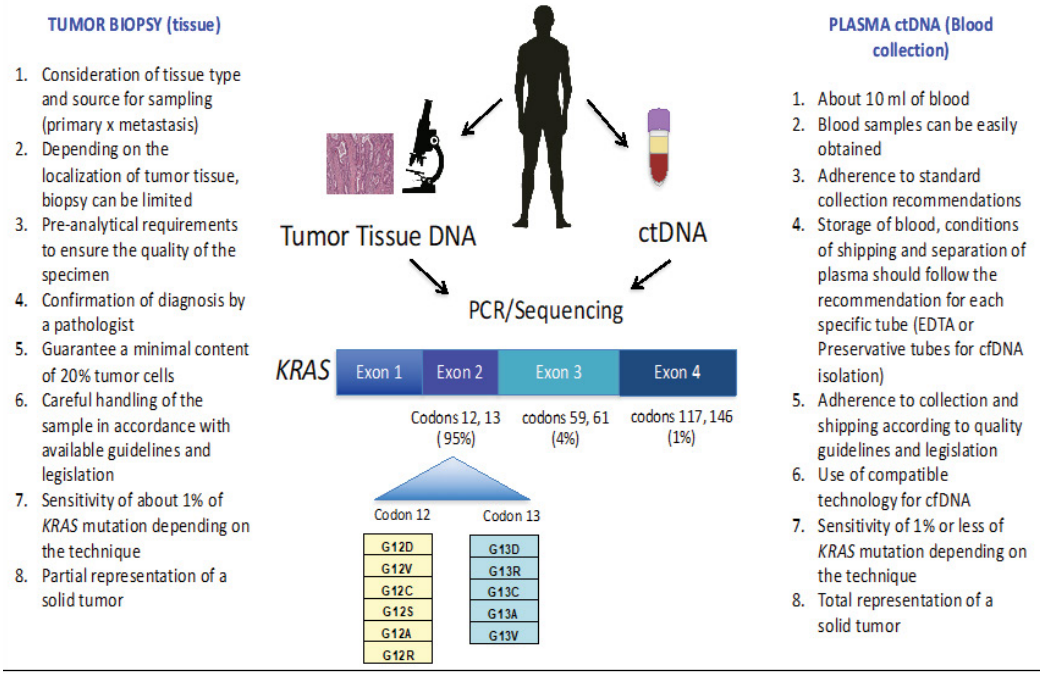


**Figure 2.** Differences in care and use of tissue biopsy and liquid biopsy for *KRAS* testing. Comparison of liquid and tissue biopsy for molecular testing.

Plasma is the most frequent body fluid used for liquid biopsy testing. Many other body fluids can also be used such as urine, stool, cerebrospinal fluid, saliva, pleural fluid, and others for specific conditions [62].

Molecular testing has developed from single target testing to the possibility of multi-target analysis. For example, the development of Next-Generation Sequencing-Based (NGS) technologies has facilitated the study of the genome at a broader scale than previously possible [63]. ctDNA is most commonly used to detect point mutations such as *EGFR, KRAS, NRAS, BRAF* and *PIK3CA*, for which there are clinically approved targeted therapies. The evolution of technology makes it now possible to detect multiple genomic alterations (mutations, indels, amplifications, rearrangements, microsatellite instability) by liquid biopsy.

## Tumor resistance and liquid biopsy

The information detected through liquid biopsy can be prognostic and guide therapy to overcome mechanisms of resistance, which can either be acquired during the course of the disease or induced through particular treatments. One example of resistance-acquired mutation is T790M in the *EGFR* gene, which can arise in patients treated with first generation TKIs. The identification of this mutation led to the development of third generation TKIs that showed clinical benefits for patients with lung cancer resistant tumors [64]. Using liquid biopsy for monitoring this mutation in lung cancer patients in Brazil has been shown as cost effective [65]. Also, liquid biopsy overcomes re-biopsing issues in patients that otherwise would not have any material to be analysed upon progression, allowing for identification of resistance mutations.

CRC is another cancer for which liquid biopsies will be used more often in the near future, as it has been demonstrated that ctDNA is detected in almost all patients [66]. Screening for mutations in *KRAS* has been mandatory for metastatic CRC patients to determine if they would benefit from monoclonal antibodies (MoAbs) inhibiting the EGFR-activation cascade, such as cetuximab and panitumumab. Patients whose tumors are *KRAS* wild type (wt*KRAS*) are eligible for these MoAbs, while patients with KRAS mutation are not. For *KRAS* testing, the use of ctDNA may provide an alternative when tissue-based testing is technically or logistically unfeasible.

Despite some concerns about reliability of adopted techniques and the reproducibility of the findings, many studies have been exploring its clinical use during both disease presentation, but more importantly at progression [67]. wt*KRAS* patients undergoing EGFR therapy may become resistant due to acquired *KRAS* mutations [68]. This mutational status can be used to define if patients who failed treatment with anti-EGFR therapy could be re-challenged with another EGFR inhibitor.

Many other applications for distinct types of cancer have been studied and many ongoing trials have the potential for generating breakthrough findings that will impact the current dynamics and result in a faster adoption of liquid biopsy than expected.

## KRAS Activating mutation and KRAS G12C inhibitor

*KRAS* mutations are detected in about 20% of human cancers [69]. The highest incidence of *KRAS* mutation is in codon 12, where Glycine might be changed to Aspartate (p.G12A), Alanine (p.G12D), Cysteine (p.G12C) or Valine (p.G12V). The differences in *KRAS* codon 12 mutations may lead to different clinical outcomes in patients who may be progressing.

In CRC, *KRAS* p.G12V or p.G12C mutations may be independent prognostic factors in patients with stage I-III CRC [70]. In a retrospective analysis, p.G12C and p.G12V mutations were independently associated with worse overall survival after diagnosis of advanced and recurrent CRC [71]. In patients who recurred after resection of colorectal liver metastases, p.G12V, p.G12C, and p.G12S mutations were associated with worse overall survival (OS) [72]. Finding these distinct subgroups of patients with worse prognosis can be the first step in finding specific therapies targeting these mutations, potentially improving those patients prognosis.

In lung cancer, presence of *KRAS* p.G12C mutations influence patients' prognosis, response to targeted therapy and chemotherapy, with worse OS [73-75]. A study evaluating a subgroup of *KRAS*-positive patients receiving EGFR-TKI therapy showed that patients harboring p.G12C displayed decreased progression free survival (PFS) when compared to non-G12C patients, but no difference in OS was observed. These data indicate that p.G12C *KRAS* mutation as a strong negative predictor for EGFR-TKI treatment, mirroring the result already validated for CRC [76]. As p.G12C is the most frequent alteration found in smoking-related lung cancer, it is expected that drugs targeting this molecular alteration will positively affect the landscape of lung cancer treatment. These subpopulations with different *KRAS* mutation types should be considered as different subgroups for optimal regimen selection.

For over four decades, exhaustive efforts were made to find effective pharmacologic inhibitors for KRAS onco-proteins due to the high frequency of this alteration [77]. Several strategies to inhibit KRAS oncogenic signaling has

been attempted throughout the years where early approached included indirect inhibition of either the KRAS subcellular localization or KRAS downstream effectors with no success. Nonetheless, promising KRAS inhibitors are under investigation and a drug that directly inhibits mutated kras proteins with a predicted higher affinity for p.G12C mutations is being tested. Several clinical trials are currently recruiting patients with locally advanced or metastatic malignancy with *KRAS* G12C mutation identified. Promising preliminary results are being demonstrated with this strategy. For a full list of ongoing clinical trials, please refer to Table 2.

*KRAS* screening is included as part of standard molecular testing for advanced lung cancer in international guidelines [78]. *KRAS* mutation has been associated with poor overall survival of lung adenocarcinoma patients in several studies, including one conducted with Brazilian patients [28,79,80]. Also, because of the promising efficacy of *KRAS* inhibitor that binds to *KRAS* G12C, the interest in screening for *KRAS* in NSCLC is growing.

Frequency of *KRAS* G12C mutation widely varies among tumors. Worldwide, *KRAS* G12C mutation rate varies from 4% and 12% for CRC and NSCLC respectively [81]. As shown in Table 1, the frequency of G12C in the Brazilian population varies from <1% to 11.3% in CRC and 3.4% to 25.4% in lung. In pancreatic cancer, a small study (n = 23) did not find any tumors with G12C mutation, maybe because of the size of the sample.

## ctDNA current and future application

ctDNA analysis has various applications for monitoring treatment response and guiding treatment choice and, so far, is not used as a diagnostic tool in standard clinical care. Experimental studies have shown promising results in favor of using liquid biopsy for diagnosis in the future [82]. ctDNA for detecting known somatic mutations has been used to monitor minimal residual disease in research settings [83]. This applicability can be very useful and has significant potential for anticipating relapse. Another application for ctDNA is to monitor therapy response, providing support for clinical decision making and aiding in the prescription of targeted therapy. Quantitative approaches using the alteration of the allelic fraction of mutations, reflects tumor burden of an individual patient [84]. This evidence supports ctDNA as a reliable tool for monitoring treatment response whenever serial testing is required.

## Barriers to KRAS testing in Brazil

Barriers to *KRAS* testing include all of those related in the process of molecular testing, from sample extraction to results interpretation. Despite technological advances, gaps in knowledge about molecular testing remain among the scientific community. These gaps include lack of basic understanding of its concept, application, results interpretation and the reliability of specificity and sensitivity of the tests. Additionally, there is currently no regulation related to molecular testing in the country.

Given Brazil's size and inherent inequalities, there is uneven distribution of technology, expertize and facilities to complete quality local molecular analysis. This maldistribution leads to major logistical barriers. Furthering these inequities, a sizeable gap in training of incoming and existing personnel, including pathologists, exists throughout the country. Also, there is not a

**Table 2.** Ongoing clinical trials for *KRAS* testing.

| Description | Neoplasy | Treatment Arms | Primary Endpoint | Phase | Planned Patients | Status | Starting Date | Estimated Completion Date | Trial Identifier |
|---|---|---|---|---|---|---|---|---|---|
| JNJ-74699157 | KRAS G12C Part 1: Advance/ Metastatic Solid Tumors Part 2: NSCLC, CRC | Part 1: Dose Escalation Part 2: Dose Expansion | DLT, ORR, % AEs, RP2D | I | 140 (only 10 patients) | Completed | July 26, 2019 | July 13, 2020 | NCT04006301 |
| MRTX849 | KRAS G12C Advance/Metastatic Cancer | Phase 1: Dose Exploration Phase 1b: Expansion Phase 2: ORR | DLT, ORR | I/II | 391 | Recruiting | January 15, 2019 | September 2022 | NCT03785249 |
| LY3499446 | KRAS G12C Advanced Solid Tumor, NSCLC, CRC | LY3499446 Phase 1 Phase 1: LY3499446 + Abemacicilib Phase 1: LY3499446 + Cetuximab Phase 1: LY3499446 + Erlotinib LY3499446 Phase 2 Phase 2: LY3499446 + Abemacicilib Phase 2: LY3499446 + Cetuximab Phase 2: LY3499446 + Erlotinib Phase 2 Active Comparator: Docetaxel Phase 2 | DLT, ORR, PFS | I/II | 230 | Closed due to toxicity | November 27, 2019 | December 6, 2021 | NCT04165031 |
| JNJ-74699157 (ARS-3248) | Neoplasms, Advanced Solid Tumors, NSCLC, Colorectal Cancer | Part 1: Dose Escalation Part 2: Dose Expansion | DLT, AEs | I | 10 | Completed | July 26, 2019 | July 13, 2020 | NCT04006301 |
| AMG 510 (CodeBreaK100) | KRAS p.G12C Mutant Advanced Solid Tumors | Dose Exploration Part 1 monotherapy Dose Expansion Part 2 monotherapy Phase 2 monotherapy Combination arm with AMG 510 and anti PD-1/ L1 Monotherapy treatment naive advanced NSCLC | Number of participants with abnormal laboratory values and changes in vital signs and ECG, ORR | I/II | 533 | Recruiting | August 27, 2018 | February 14, 2024 | NCT03600883 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| AMG 510 (CodeBreaK101) | Advanced Solid Tumors, Kirsten Rat Sarcoma (KRAS) pG12C Mutation | Sotorasib + MEK inhibitor<br>Sotorasib + SHP2 allosteric inhibitor<br>Sotorasib + Pan-ErbB tyrosine kinase inhibitor<br>Sotorasib + PD-L1 inhibitor<br>Sotorasib + EGFR inhibitor +/- Chemotherapeutic regimen<br>Sotorasib + PD-1 inhibitor<br>Sotorasib + Chemotherapeutic regimen<br>Sotorasib Monotherapy<br>Sotorasib + CDK inhibitor<br>Sotorasib + mTOR inhibitor<br>Sotorasib + MEK inhibitor + EGFR inhibitor | Number of participants with abnorml laboratory values and changes in vital signs and ECG | I | 1,003 | Recruiting | December 17, 2019 | July 4, 2027 | NCT04185883 |
| AMG 510 Ethnic Sensitivity Study (CodeBreaK105) | Advanced/ Metastatic Solid Tumors With KRAS p.G12C Mutation | Sotorasib | Number of participants with abnormal laboratory values and changes in vital signs and ECG | I | 12 | Recruiting | April 28, 2020 | March 5, 2022 | NCT04380753 |
| AMG 510 Proposed INN Sotorasib (CodeBreaK200) | KRAS p, G12c Mutated/Advanced Metastatic NSCLC | Sotorasib vs. chemotherapy (docetaxe) | PFS | III | 650 | Recruiting | June 4, 2020 | July 1, 2029 | NCT04303780 |
| MRTX849 in Combination With TNO155 | Advanced Solid Tumors With KRAS G12C Mutation KRYSTAL 2 | Phase 1 Dose Exploration<br>Phase 1b Expansion<br>Phase 2 | Treatment-related AEs; Adagrasib blood plasma concentration | I, II | 148 | Recruiting | April 22, 2020 | October 2022 | NCT04330664 |
| GDC-6036 | NSCLC; Colorectal Cancer<br>Advanced Solid Tumors | Dose-escalation (Stage I), Dose Expansion (Stage II)<br>GDC-6036 + Atezolizumab (Stage I and Stage II)<br>GDC-6036 + Cetuximab (Stage I and Stage II)<br>GDC-6036 + Bevacizumab (Stage I and Stage II)<br>GDC-6036 + Erlotinib (Stage I and Stage II) | AEs, DLTs | I | 236 | Recruiting | July 29, 2020 | August 31, 2023 | NCT04449874 |

**Table 3.** Minimal requirements for *KRAS* molecular testing.

| Tumor Biopsy | Plasma ctDNA (Blood collection) |
|---|---|
| Multi-departmental consideration of tissue type and source for sampling | About 10 ml of blood |
| KRAS testing should be repeated after all tumor recurrence, especially when considering targeted therapy | Adherence to standard collection recommendations |
| Pre-analytical requirements to ensure the quality of the specimen | Adherence to correct shipping and processing, conditional to the tube type used for sampling and shipment (The storage of blood, conditions of shipping and separation of plasma should follow the recommendation of each tube–EDTA or Preservative tubes for cfDNA isolation) |
| Confirmation of diagnosis by a pathologist | Compatible technology for cell-free DNA |
| Guarantee a minimal content of 20% tumor cells | Know that therapy can drastically interfere in amount of tumor DNA circulating in the plasma. |
| Careful handling of the sample in accordance with guidelines and legislation | Adherence of the guidelines and legislation with the shipping of the blood tube |
| Optimize the use of the paraffin-embedded tissue | |

national institution that provides accreditation for molecular testing, leading to differences in test results. Government policy makers, ANS, relevant Medical Societies and other affected stakeholders, should establish guidelines based on the medical need and the science.

While costs of molecular testing are declining and these tests are becoming more available worldwide, it is not affordable for most of the Brazilian population. Relying on out-of-pocket payments for testing is a significant barrier to access appropriate treatment. A specific payment procedure for *KRAS* testing is not defined which creates a miscommunication between payers and labs, resulting in a lack of reimbursement.

Although lack of access may only impact CRC at this time, this problem will continue to compound in parallel with the increasing development of targeted therapies requiring this test. These new companion therapies will require more tests and, therefore, an absence in widespread adoption of KRAS testing will lead to major setbacks in specific and appropriate treatment for patients who may need it.

This panel has addressed the lack of access and barriers to the current adoption of *KRAS* testing in Brazil. Lack of access to testing is not unique, and the specific issues discussed are not exclusive to this country. But we have enough reasons to think we are ready to implement this technology. Hence, these recommendations could be useful for Brazil and other countries experiencing similar issues (Table 3). With increasing costs of oncology diagnosis and treatment, recommendations stated here can serve as a framework for countries in the early phase of molecular testing adoption.

## Conclusion

*KRAS* testing should be ordered only when results will guide available therapy. In Brazil, although *KRAS* testing is currently restricted only for metastatic CRC patients, the use of this type of testing in lung and pancreatic cancer shows great promise, since new drugs are being developed to treat specific *KRAS* mutated tumors. These potential therapies highlight an increased importance of this technology in the future as testing requests rise. Therefore, besides addressing the aforementioned barriers for molecular testing in the country, the panel recommends the following to ensure prime quality *KRAS* testing:

1. *KRAS* testing can be used as a single gene test or as part of a larger multi-gene panel.
2. As multi-gene panel analysis is expanded, a mutated *KRAS* result should not guide therapy as an agnostic marker.
3. Codons 12, 13, 59, 61, 117, and 146 should always be evaluated when *KRAS* testing is performed.
4. Involvement of a pathologist in all stages of the tissue related procedures is recommended.
5. The technology used should be able to differentiate among mutations within each codon.
6. *KRAS* status can be assessed in tumor cells when using tissue or ctDNA when using liquid biopsy if tissue is not available.
7. A negative result from liquid biopsy does not exclude the presence of mutations in the tumor, therefore in these cases, a tissue biopsy or the repetition of the liquid biopsy should be considered.
8. All pathology labs should follow the pre-analytical requirements to ensure the quality of the specimen.
9. Labs performing this test should follow best practices in molecular testing in both tissue and liquid biopsy and take part in External and Internal Quality Control Programs when available.
10. Careful handling of the sample in accordance to available guidelines and legislation should be ensured.
11. A user-friendly report on the results of the test should be available in the local language.
12. Interdisciplinary consideration of tissue type and source for sampling for *KRAS* testing should be implemented.

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## Conflicts of Interest

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## Author Contributions

All authors participated and made significant contributions to the data search, drafting, and discussion of the topic and all subtopics provided in this manuscript.

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