THE CHALLENGES AND PERSPECTIVES OF LIQUID BIOPSY IN BRAZIL: A CRITICAL REVIEW AND RECOMMENDATIONS

REVIEW

HIGHLIGHTS

- Liquid biopsy provides a useful, accessible and cost/effective tool for cancer management in Brazil
- Liquid biopsy complements current cancer management especially given tumor heterogeneity, evolution over time and response to treatment
- Current approved clinical guidelines include liquid biopsy in lung cancer, however the potential for screening, early detection, risk of relapse, cancer management in other tumor types is enormous

ABSTRACT

Cancer is the second cause of death in Brazil and there are significant disparities in the quality of the services offered to cancer patients in the country. These disparities are observed through the continuum of cancer care and significantly affect patient outcome and disease burden. Due to the high specificity and sensitivity, relative low costs and straightforward analyses, liquid biopsies based on ctDNA plasma detection have been used worldwide and considered as a viable and promising tool in cancer management. Herein, the authors provide a critical review about the current status and perspectives of liquid biopsy usage in Brazil, the challenges for its broad clinical use and its pros and cons, such as the usefulness in cancer diagnosis, selection of molecular targets, assessment of the patient's response to therapy, development of therapy resistance and detection of minimal residual disease. Finally, the authors propose a series of recommendations specific to Brazil, a large country with limited and unequal distribution of health resources, to increase the use of liquid biopsies for cancer management, thereby improving access to this new molecular tool.

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INTRODUCTION

Modern management of cancer includes not only clinical, surgical and radiological tools, but also requires molecular evaluation of the tumor. There are several therapeutic advances in oncology treatment due in part to a more complete understanding of its genomic portrait, allowing precision medicine to treat the right patient at the right time with the right therapy [1]. Although tumor biopsies remain the gold standard for cancer genotyping, these do not capture the entire molecular landscape of the tumor. Liquid biopsy has emerged as a powerful complementary tool to personalize cancer care. This paper reviews the current state of liquid biopsy in Brazil, its current and future applications, the challenges for clinical use, and makes recommendations to increase its wider adoption.

METHODS

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 the field of precision medicine and companion diagnostics. Pub Med and Embase were used to identify clinicians and scientists with an academic or hospital affiliation, and who had published in the field of precision medicine and companion diagnostics since 2010. Augmenting this search, AHF contacted numerous other individuals in various countries in Latin America and elsewhere 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 oncology, pathology, genetics and applied genomics. Great attention was paid to ensure a diverse group representing various disciplines related to precision medicine and companion diagnostics.

> *Search Strategy and Selection Criteria*
> Papers useful for the consensus discussion and the references cited in this paper were identified through searches of PubMed and Embase with the search terms "precision medicine", "targeted therapy", "targeted oncology therapy", and "companion diagnostics" from 2010 until 2018. 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 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 to the meeting, the Panel was asked to review the document and to again acknowledge that they were in full-agreement.
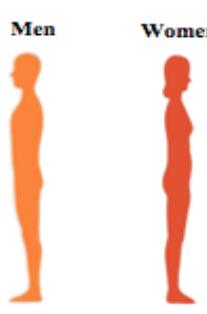
DISCUSSION

Brazil is the largest country in Latin America and fifth in the world, with an estimated population of more than 208 million. Continued population growth is expected for at least the next 30 years - by 2050, the Brazilian Institute of Geography and Statistics (IGBE) projects a population of 238 million. However, significant changes to its demographic make-up is also expected. IGBE projects the elderly's share of the population to reach 23.8% by the mid-21st century (66 million people above the age of 60), inverting the current elderly-to-young ratio [2, 3]. This rapid ageing trend constitutes one of the toughest challenges facing Brazil, particularly with respect to its burdens on the health system (cancer, chronic diseases and disabilities) and social security [2, 3]. Moreover, as in many other Latin American countries, income disparity is high despite overall GDP growth over the past decade, further stressing funding capacity for the larger, poorer portion of the population.

Brazil has a public health system that aims at providing healthcare to the entire population [4]. However, about 25% of the population relies on insurance coverage and access to the private health system. Significant discrepancies between the services offered in and within both systems are observed impacting cancer care [5, 6]. For instance, a retrospective breast cancer study conducted in Brazil highlighted that patients in the public health system are diagnosed with a more advanced disease and have worse overall survival rates when compared with those in the private system [7].

From a public health standpoint, cancer represents a significant share of the burden of disease on the country's population. Cancer is the second cause of death in Brazil [8] with an incidence rate calculated as 205.5 per 100,000 people/year, resulting in approximately 634,880 newly diagnosed cases in 2018 (51%

in men and 49% in women) [9, 10]. As seen in Figure-1, the cancer types in Brazil with the highest incidence are prostate, lung, colorectal and stomach in men, whereas in women the most frequent cancers are breast, colorectal, cervical, lung and thyroid.

Primary Location	Cases	%
Prostate	68.220	31.7
Lung	18.740	8.7
Colon/rectum	17.380	8.1
Stomach	13.540	6.3
Oral cavity	11.200	5.2
Esophagus	8.240	3.8
Bladder	6.690	3.1
Larinx	6.390	3.0
Leukemia	5.940	2.8
Central nervous system	5.810	2.7

Men   Women

Primary location	Cases	%
Breast	59.700	29.5
Colon/rectum	18.980	9.4
Uterine cervix	16.370	8.1
Lung	12.530	6.2
Thyroid	8.040	4
Stomach	7.750	3.8
Uterine body	6.600	3.3
Ovary	6.150	3
Central nervous system	5.510	2.7
Leukemia	4.860	2.4

Figure 1. Distribution of the ten cancer types of highest incidence estimated for 2018 by gender, excluding non-melanoma skin. *(Adapted from: https://www.inca.gov.br/numeros-de-cancer)*

However, gaps and inequities in access to quality and effective diagnostic services and treatment exist. For instance, access to specialized health services in the country for cancer patients varies depending on their geographical location. Approximately 72% of diagnostic support services are provided by private laboratories located in the south and southeast regions of the country [11]. These laboratories are usually associated with comprehensive cancer centers located in urban areas further, limiting the access to appropriate care for patients in other regions.

Additionally, access to molecular testing is still limited in Brazil particularly in the public health care system [1, 11]. A study by the Brazilian Group of Thoracic Oncology (GBOT) showed that the majority of lung cancer patients are not tested for epidermal growth factor receptor (EGFR) mutations and the majority of these tests are performed in private laboratories [12]. More recently, in vitro diagnostic devices or next generation sequencing platforms, that analyze hundreds of genes simultaneously, have been incorporated in tumor molecular profiling in some private laboratories. However, these tests are costly and labor intensive, limiting their use, particularly for patients in the public system.

Reliable and complete epidemiological data are still lacking, limiting public policy formulation. Cancer burden statistics in Brazil provided by the Brazilian National Cancer Institute (INCA) [9] are estimates and do not provide staging data [13], which are critical components for effective planning for cancer control programs and policy implementation. The lack of adequate and accurate data also limits the assessment of the impact of new technologies to control and prevent cancer in the country, hampering even further the timely implementation of new technologies such as liquid biopsy, regardless of the its proven usefulness in cancer care management.

Disparities in cancer incidence and prevalence rates within Brazil's regions, as well as access to timely and effective diagnostics and treatment, are reflected in projected trends in the disease burden. In Brazil, prediction of cancer mortality trends for 2030 estimate that the north and northeast regions of Brazil will have increases in mortality cancer rates, whereas reductions are predicted for the remaining geographic regions [8]. Analysis of the epidemiological situation and logistic limitations with an understanding of ways to leverage existing provider networks (particularly via payers) in each region of the country, coupled with innovative ways to communicate and refer patients in need of better diagnostic tests from areas with significant gaps in access, are required to improve the development of future public health policies.

As in other countries, cancer significantly impacts healthcare sector finances in Brazil. The total cost of cancer care was estimated around USD $59.7 billion in 2015, which represented 1.7% of the GDP in that year and continues to grow. The total cost of cancer in Brazil is rising exponentially and is predicted to reach nearly USD $81 billion in 2020 [14]. Based on International Agency on Cancer Research (IARC) data, the Brazilian Society of Pathology (SBP) estimates that the financial loss caused by cancer deaths alone (i.e., excluding treatment-related costs) exceeds USD $9 billion annually. However, investments on cancer diagnosis remains modest in Brazil. Cross analyzing the IARC data with the public health system investments in lab tests, SBP projected that the financial burden of cancer treatment was 200 times more than all governmental investments on cancer diagnoses in 2017 [15].

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Consequently, researchers and policy makers have been striving to develop more cost-effective methods for diagnosing, treating and managing cancer. Liquid biopsy is considered a viable and promising tool in cancer management worldwide [16].

LIQUID BIOPSY

Liquid biopsies are diagnostic methods based on the detection of circulating tumor material such as cells, nucleic acids, proteins and extracellular vesicles in a minimally invasive or non-invasive manner through the sampling of blood or other body fluids [17]. However, the detection and analysis of circulating tumor DNA (ctDNA) is proving to be the most clinically useful because of its high specificity and sensitivity, relative low cost and straightforward analysis. For the purpose of this paper, we are focusing on ctDNA liquid biopsy.

The term ctDNA refers to tumor-derived DNA fragments present in the circulation of cancer patients and should not be confused with circulating cell-free DNA (cfDNA), a broader term used to refer to all free DNA fragments present in the blood stream, but not necessarily originated from tumor cells. Apart from plasma and serum, ctDNA can be isolated from saliva, cerebrospinal, prostatic, lymphatic and peritoneal fluids, bronchial lavage, sputum, gastric and biliary juices, urine and stool samples. ctDNA is mostly double stranded and highly fragmented. It is thought to originate through apoptosis and cell necrosis, and possibly also through active secretion in small extracellular vesicles. Another important property of ctDNA is its rapid turnover. The proportion of ctDNA in plasma from cancer patients can range between 0.01% and 93% of total cfDNA [18, 19]. This high variability can be explained by biological differences such as tumor location, histology, vascularization, volume and stage. Variation can also be explained by the different sensitivities and specificities of the analytical methodologies employed for ctDNA detection and also by the presence of contaminating high molecular weight DNA [20, 21].

In a comprehensive study, Bettegowda and colleagues used digital polymerase chain reaction-based (dPCR) technologies to measure levels of ctDNA with tumor-specific DNA mutations or structural rearrangements in plasma samples from 640 patients with various cancer types. The results showed a wide variability in the proportion of metastatic patients with detectable ctDNA in plasma. Among study participants with advanced metastatic disease, ctDNA was detected in 82% of patients with solid tumors outside the brain, including more than 75% of those with advanced ovarian, colorectal, bladder, gastroesophageal, pancreatic, breast, hepatocellular, head and neck cancers, and melanomas. However, the authors detected ctDNA in less than 50% of patients with medulloblastomas or metastatic cancers of the kidney, prostate or thyroid. Among patients with stage I cancer of all types, approximately 45% had detectable ctDNA, whereas the proportion of patients with detectable ctDNA increased progressively according to the tumor stage. Additionally, a 100-fold increase in the median ctDNA concentration was observed in patients with advanced disease when compared to patients with early disease stages [22].

Due to its low concentration in the circulation and high degree of fragmentation, ctDNA detection is challenging and requires highly specific and sensitive methodologies. Broadly, current approaches for ctDNA detection can be divided into two categories: 1) targeted approaches that include the analysis of single or few known genetic changes in actionable genes, and 2) global approaches which allow the detection of tumor-specific DNA alterations, without knowledge of any specific changes present in the primary tumor [23]. The limit of current assays for ctDNA detection is 0.1% (1 mutated tumor-derived molecule within 1,000 wild type molecules) and specificity has generally been shown to be higher than 95% [24].

Liquid biopsies have several advantages over tissue biopsies but have some hurdles yet to overcome. The advantages and disadvantages of liquid biopsy over tumor tissue biopsy are summarized in Table-1.

Type of biopsy	Advantages	Disadvantages
Tissue Biopsy	Lower costs	Often difficult and invasive
	Reimbursement	Multiple sampling is difficult
	Site specific	Single snapshot over time and space
	Histological diagnosis, tumor staging and IHC[1]	Not representative of the genomic tumor landscape
	Minimally invasive procedure	Requires high end technology
Liquid Biopsy	Lower morbidity	Not yet reimbursed
	Easily repeatable and highly reproducible	Not site specific for cancer
	Allows real-time monitoring of disease	Does not allow tumor histological characterization and tumor staging
	Better represents tumor genetic heterogeneity and burden	May not differentiate between malignant and non-malignant conditions[2]

[1]Immunohistochemistry, [2]Systemic inflammatory disease, infections, trauma, autoimmune disease and benign tumors.

Table 1. Comparison between Tumor Biopsies and Liquid Biopsies.

Tumors are genetically heterogeneous and evolve over time creating a challenge to capture information by a single tissue biopsy. Additionally, during the course of cancer treatment, resistance-associated mutations may develop as a result of selective pressure, changing the response to therapy and requiring restaging. In this way, liquid biopsy allows a longitudinal evaluation of cancer-related mutations and those new mutations related to resistance of targeted therapy.

Moreover, liquid biopsies are capable of detecting mutations in cases where the number of tumor cells is insufficient for molecular tests. Also, liquid biopsy is a better option for patients for whom invasive sampling is contraindicated or the tumor is difficult to be sampled. Conversely, liquid biopsy does not allow histological characterization of the tumor, and the clear distinction between benign, pre-malignant and malignant lesions is not possible thus far.

Therefore, after reviewing the advantages and disadvantages between liquid biopsy and tissue biopsy, the former represents a complementary approach that captures tumor genomic information using circulating DNA, which is required in the modern approach of cancer treatment.

USES OF LIQUID BIOPSY
There are a number of applications for liquid biopsy, but the only proven uses are to monitor disease response and detect the emergence of drug resistance in certain tumor types, such as lung, colorectal and breast cancers. Currently, the clinical guidelines recommending the use of liquid biopsy are only for the use in lung cancer management. Other uses are currently being reviewed and future potential is vast. Within these are cancer screening and early detection of disease, the use of liquid biopsy as a companion diagnostic tool, monitoring of tumor burden and minimal residual disease. However, there is sufficient evidence in the literature to support its use in other tumor types [25 – 30].

Screening and early cancer diagnosis
The importance of screening and early detection is the high impact it has on survival rates. Liquid biopsy can potentially contribute to earlier detection of tumors that currently do not have efficient screening methods for the general population. For the general population, there are currently no studies that support the use of liquid biopsy as a cancer screening tool. However, ctDNA can be detected with high sensitivity and specificity in patients with early stage disease suggesting liquid biopsy can be used as a screening tool in the future [31]. For high-risk populations, such as the ones with hereditary predisposition to cancer, or with chronic exposure to toxic agents, liquid biopsy has the potential to increase early detection.

Recent proof-of-concept studies have demonstrated that ctDNA may be used for screening or early detection of cancer. As an example, the combination of ctDNA analysis with other biomarkers has proven particularly powerful. Cohen and colleagues screened 1,005 patients with non-metastatic cancers of the ovary, liver, stomach, pancreas, esophagus, colorectum, lung and breast. Screening tests were positive in a median of 70%, even for tumors that do not currently have effective population screening strategies. The detection sensitivity ranged from 69% to 98%, with a specificity of more than 99% [25].

In another example, ctDNA analysis of Epstein-Barr Virus was used for the early detection of nasopharyngeal carcinoma. Chan and colleagues screened 1,318 asymptomatic individuals and detected viral DNA in 69 of them, uncovering three nasopharyngeal carcinomas [32].

Additional studies should ideally screen and prospectively follow large cohorts of asymptomatic individuals to determine the real sensitivity and specificity of ctDNA for the detection of presymptomatic cancer, the rate of false-positives leading to unnecessary follow up procedures, and to evaluate whether the use of ctDNA-based tests affect patient outcomes.

Minimal residual disease monitoring and prognosis
An emergent application of liquid biopsy is the detection of residual disease during the course of treatment. The detection of ctDNA after surgery or treatment with curative intent has been correlated to prognosis in several cancer types [33 – 36]. Liquid biopsy techniques are moving precision medicine a step closer to predicting the risk of relapse in cancer patients.

For instance, Tie and colleagues prospectively followed 230 patients with stage II colorectal cancer, and the presence of ctDNA after tumor resection was correlated to a disease-free survival of 0%, as compared to 90% for the ctDNA-negative group [36]. In another study, 55 patients with early stage breast cancer receiving neoadjuvant chemotherapy were prospectively followed, and detection of ctDNA after treatment predicted metastatic relapse with a high level of accuracy, anticipating clinical relapse by almost eight months [33]. Additional studies also described that the absence of measurable ctDNA in a liquid biopsy analysis after tumor resection is associated with better patient prognosis [35, 37].

Guidance on selection of targeted therapy

Tumor genomic profiling allows the identification of molecular variants in the tumor that helps guide patient management and is incorporated as standard diagnosis procedure for certain tumor types. Currently, many different genetic biomarkers are being used for the management of cancer patients and potentially all of these genomic abnormalities can be detected by liquid biopsy [Table-2].

Cancer Type	Biomarker	Clinical Use
Breast adenocarcinoma	CTNNB1, ERBB2 (HER2), CDH1, Estrogen Receptor, Progesterone Receptor, BRCA1, BRCA2	Diagnosis Therapy guidance Prognosis
Colorectal carcinoma	KRAS/ NRAS/ BRAF/ MSI	Guide Anti-EGFR therapy Prognosis stratification Lynch syndrome screening Therapy selection
Diffuse Gliomas	BRAF, IDH1/2, 1p/19q, pMGMT methylation	Tumor diagnosis (pilocytic astrocytoma, PXA[1]) Diagnosis Drug response Therapy guidance
Endometrium cancer	PTEN, MLH1, MSH2, MSH6, PMS2	Lynch syndrome Cancer risk
GIST	KIT (CD117), PDGFRA, SDH	Diagnosis Therapy guidance
Heredit. Paraganglioma Pheochromocytoma	SDH	Diagnosis
Lung Adenocarcinoma	EGFR, ALK, ROS1, MET/ RET, BRAF	Therapy guidance
Midline Gliomas	H3F3A	Diagnosis
Melanoma	BRAF	Therapy guidance
Neuroblastoma	NMYC amplification	Prognosis
Thyroid Carcinoma	BRAF, MET/ RET	Diagnosis and screening Therapy selection and drug response prediction

[1]Pleomorphic Xanthoastrocytoma

Table 2. Partial list of molecular tests used in Integrated Diagnosis.

Decision making for targeted therapy is primarily based on the analysis of primary tumor tissue. The clinical utility of liquid biopsies for tumor genotyping is rising. Currently, there are two assays that have been approved and are in use in clinical practice. One guides the detection of EGFR mutations in non-small cell lung carcinoma (NSCLC) patients, which was approved by the FDA [38]. The other assay is designed to detect KRAS mutations in colorectal cancer and is commercially available in Europe [39]. If the tests results are positive, they can be used to guide therapy. But if the tests are negative, the patient will require tissue biopsy. Therefore, liquid biopsy is not currently used to define first line treatment, unless there is not enough tissue for tumor profiling. Both tests are available in Brazil, but lack of reimbursement is hampering their wider adoption.

Monitoring treatment response and emerging tumor resistance

Monitoring treatment response and emerging tumor resistance are the most clinically established uses of liquid biopsy, particularly in the setting of lung cancer [40]. Studies have been demonstrating that patients who develop resistance to tyrosine kinase inhibitors (TKIs) could benefit the most from liquid biopsy technologies. These studies show that ctDNA can be used in the routine management of lung, breast and colorectal cancer to supervise clonal evolution and detect treatment resistance [41], especially in patients who are treated with anti-EGFR therapy.

Given the fact that multiple target drugs now exist to treat NSCLC, liquid biopsies can greatly facilitate the longitudinal detection of resistance mutations without the need for repetitive tissue biopsies. The sensitivity of ctDNA for the detection of multiple mutations, including the EGFR T790M mutation that is associated with acquired resistance to TKIs, has ranged from 30-100%, depending on the study and the platform used, with most studies showing a sensitivity of 70-80% [40]. In this scenario, liquid biopsy results are an important complement and validated method to tissue biopsy for monitoring disease progression of patients with acquired resistant mutations [42].

The current guidelines for the use of liquid biopsy in NSCLC are [40]: 1) there is insufficient evidence to support the use of ctDNA molecular methods for the diagnosis of primary lung adenocarcinoma; 2) in some clinical settings in which tissue is limited and/or insufficient for molecular testing, physicians may use a ctDNA assay to identify EGFR mutations; and 3) physicians may use ctDNA methods to identify EGFR T790M mutations in lung adenocarcinoma patients with progression or secondary clinical resistance to anti-EGFR therapies. Additionally, the testing of the tumor sample is recommended if the plasma result is negative. These guidelines provide a helpful path for application of liquid biopsy in NSCLC, however,

application of liquid biopsy to other tumor types should be further studied and implemented.

Early recurrence detection

Numerous retrospective studies have demonstrated that disease recurrence after resection can be detected earlier with liquid biopsy than with imaging, preceding the clinical manifestations often by several months. Studies have shown that monitoring tumor-specific mutations in plasma after surgical resection allows earlier detection of disease recurrence [31, 43]. A central question to be addressed in future studies is to determine if therapeutic intervention based on molecular monitoring, with or without the support of imaging or other clinical tools, will improve the cancer patient outcomes.

OBSTACLES AND CHALLENGES OF IMPLEMENTING LIQUID BIOPSY AS A STANDARD DIAGNOSTIC METHOD IN BRAZIL

State of the art practices use integrated diagnosis, which includes pathology reports and molecular testing for some cancer types. Liquid biopsy has some technical hurdles to overcome and its utility in clinical practice has not been defined in all cancer settings. However, the potential to routinely incorporate liquid biopsy as the companion diagnostic test for cancer patients in Brazil is significant.

In general, the major technical challenges for the development of a viable approach based on liquid biopsy are related to the low concentration, instability and fragmentation of ctDNA. The amount of ctDNA varies in different patients and in different stages of cancer, limiting the sensitivity and detection of the assays. Highly sensitive techniques must be validated for detection of small amounts of ctDNA at early stages, to facilitate early disease detection. Many different liquid biopsy technologies appeared in the market in recent years, but still lack reproducible, robust, cost-effective and easy-to-use workflows.

Additionally, to perform and analyze the molecular data generated by liquid biopsies highly trained personnel and usage of high cost lab equipment are necessary, limiting the access to this new technology. These techniques may be currently accessible only at a few specialized centers. Moreover, given the fast-paced development in the area of molecular biology, there is a lack of knowledge among health care professionals and other stakeholders about the clinical utility, actual benefits and potential applications of liquid biopsy.

In brief, the major current limitations for broader use of liquid biopsy in the clinical practice in Brazil are that molecular tests are not widely available; the methodology is complex, requiring qualified technical support; the costs are still high for the majority of the population; and, as with any new technology, the role and limitations of liquid biopsy are not well known by clinicians.

It is important to consider that therapies guided by comprehensive molecular profiling do not necessarily lead to higher costs but instead optimize the usage of all treatment options available. Health economic evaluations are indispensable tools to guarantee continuity and optimal care within a financially sustainable health care system. Therefore, cost-effectiveness analyses on liquid biopsy are required to ensure wider adoption and reimbursement by all payers [44]. When these analyses can address and compare total cost of care between current diagnostic and treatment courses and those including liquid biopsy, the prospects of gaining payer interest, buy-in and coverage could be improved and achieved over a shorter period. With better access to diagnostic investigations, precision medicine then emerges as an effective alternative to allocate limited resources wisely.

RECOMMENDATIONS TO INCREASE THE USE OF LIQUID BIOPSY IN BRAZIL

Liquid biopsies have been extensively studied in the past few years. Currently, over 160 ongoing clinical trials are evaluating different applications for liquid biopsies [45]. Several applications are addressed in this paper, such as the usefulness of liquid biopsies in early cancer diagnosis, support in the selection of molecular targets, assessment of the patient´s response to therapy, development of therapy resistance, and detection of minimal residual disease.

This group of experts has reviewed the current and potential uses of liquid biopsy in Brazil, and the challenges for its wider implementation with a profound understanding of the particularities and country settings. As a result, we emphasize in the following recommendations to increase the use of liquid biopsy in Brazil:

1. Develop general guidelines on the uses and applications of liquid biopsy in clinical practice. Apply current international guidelines available for specific cancers and layout the process to create/implement the guidelines as they become available in other tumor sites. The current applications must be included as a general practice in all training settings given its demonstrated

benefit in monitoring treatment response and emerging tumor resistance.

2. Establish best practices for liquid biopsy processing, including standardized protocols for sample collection, processing and ctDNA detection. Best practices should be developed to decrease the burden of methodological complexities and ensure a highly reliable standardized process. Of most importance, the result of the best practices must put forward a method that is easily reproducible, with the highest quality to ensure the best results.

3. Engage payers in ways that their own goals are addressed, such as improved member health outcomes and reduced medical risk and cost, by: 1) developing cost-effectiveness analyses that reveal the benefits of covering liquid biopsy testing to increase its use and 2) leveraging provider networks´ potential to guide appropriate patients toward liquid biopsy testing.

4. Increase liquid biopsy clinical trials in Brazil. Given the potential breakthroughs of liquid biopsy, the large population and the burden of cancer in the country, public and private research groups have the opportunity to collaborate and test the advantages of liquid biopsy as a screening method following large cohorts of individuals.

5. Strengthen the Brazilian Cancer Registry to include current patient data and staging information. This will not only help improve the adequate measurement of treatment outcomes, but it will facilitate the analysis of public policy implementation related to cancer screening, burden, treatment and outcomes. Additionally, data should be supported by strong bioinformatics platforms and infrastructure that need to be developed by academics and researchers with public or private funding. Quality and accurate cancer data will also help foster health economic studies to evaluate the value of liquid biopsy based on outcomes and quality of life, building the case for payers to reimburse it.

6. Train health professionals, technicians and students on methodology, clinical implication and cost effectiveness of liquid biopsies and implement continued medical education programs focused on the appropriate use of this technology in the clinical practice.

7. Encourage health professional associations and patient advocacy groups to engage the government to include liquid biopsy procedures as a viable complimentary diagnostic tool.

8. Increase public awareness of the use and benefits and limitations of liquid biopsies. The general public and the media, including health journalists should be informed of the outstanding breakthroughs liquid biopsy is bringing to cancer management. Public awareness and accurate information will contribute to inform that liquid biopsy is not a panacea for cancer diagnosis but a complementary tool in cancer monitoring, early diagnosis and patient management.

CONCLUSIONS AND FUTURE DIRECTIONS

In this consensus, the current Brazilian health system in relation to the burden of cancer management as a scenario for the adoption of liquid biopsy as a disruptive new tool for management of the disease is reviewed. Liquid biopsy is being used as a complementary diagnostic tool in cancer management and the current uses of liquid biopsy include monitoring of disease response and detection of drug resistance in certain tumor types. Additionally, the clinical potential for screening, early diagnosis, selection of targeted therapy, tumor burden and residual disease detection are currently being reviewed and are showing very optimistic results. For a country such as Brazil, with a large and aging population, a high burden of cancer, an inequitable health system, financial constraints and regulatory limits, liquid biopsy emerges as a reliable, cost-effective and accessible tool to include in cancer management. However, implementation of new technologies takes time, involves highly trained personnel and equipment for testing and interpretation, and requires clinicians to be up-to-date with fast paced and continually evolving research. However, given therapies guided by comprehensive molecular profiling do not necessarily lead to higher costs but instead optimize the usage of all treatment options available, the consensus agreed to continue moving forward the discussion to increase wider adoption of liquid biopsy in Brazil.

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