




# Implementing somatic mutation testing in clinical setting: recommendations from a panel of experts.

Implementando testes de mutação somática em ambiente clínico: recomendações de um painel de especialistas.

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## ABSTRACT

There has been a rapid increase in the volume of genomic data gathered from different cancers, this has helped to develop new tumor classifications as well as to select better tailored therapies for the patients. Some of the genomic markers identified are also prognostic and predictive factors. Additionally, many technologies have been used to investigate these alterations, each with different benefits and caveats. The Genomics Committee from the Sociedade Brasileira de Oncologia Clínica (SBOC) put together a group of specialists, from different regions of Brazil that work both in the private and public scenario, to gather and organize the information regarding the utility of somatic mutation testing in solid tumors. This special article summarizes their recommendations on how to better incorporate this information into clinical practice.

**Keywords:** Somatic mutation testing, Comprehensive genomic profiling, Genomic medicine, Precision oncology.

## INTRODUCTION

Genomic Medicine is an emerging area of medicine that is characterized by the use of data derived from an individual's DNA and that is part of both his medical follow-up (disease prevention, diagnosis, or therapeutic decisions) and health policies. This concept was adapted from the Genomic Medicine Working Group, a working group developed by the North American NIH (National Institutes of Health) to increase the speed of translating data obtained in the laboratory to clinical practice.

Genomic medicine is part of a broader concept of individualized medicine, called Precision Medicine. The Precision Medicine includes new imaging tests, with or without radionuclides, nanotechnology, and the assessment of non-nucleic biomarkers in body fluids and tissues (<https://www.genome.gov/health/Genomics-and-Medicine>).

This consensus refers to somatic mutational panels, investigated in nucleic acids from tumor tissues. Platforms that evaluate gene expression and methylation tests are not discussed here. Analyses of somatic mutations in circulating tumor DNA can be discussed in an exploratory way, but they are not part of the scope of these recommendations.

### 1.1 Genomic Medicine and Oncology

Nowadays, the oncologist is faced with an enormous amount of data from discoveries about the variants in the individual structure of DNA and RNA molecules. These acquired changes can be associated with different phenotypes in the tumor cell, resulting in changes in the behavior of them. We know that the integration of this knowledge in clinical practice has already started and it is increasing. The fact itself is not surprising, since several steps in the process of fighting cancer can be decoded with greater precision from the data of the tumor genetics.

Cancer epidemiology, prevention, risk, prognosis, and therapeutic decision are already affected by the

knowledge from studies of cancer genomics. The challenge is to educate the clinical oncologist about how these data are obtained, how it is validated, and how it impacts the clinical practice.

Despite the rapid progress in the area, there are many challenges to be faced in the coming years: the lack of familiarity with this technology amongst patients and doctors, which can result in resistance to use it; the small number of professionals trained in genomics; the scarcity of specialists and bioinformatics laboratories; the difficulty of access, and the cost of exams. The faster rate that the potentially relevant genetic data are obtained without the previous discoveries having yet been assimilated is another difficult.

The disproportion of data about cataloged genetic variations in populations in some regions of the world in comparison to developing countries, with very diverse ethnicities, is another obstacle that needs to be overcome in the coming years. Finally, a practical aspect still delays the implementation of genomic medicine on large scale in oncology: the evidence is still limited on the efficacy and cost-effectiveness of genomic studies on large scale, except for some mutations clinically validated in a small group of genes.

Therefore, SBOC decided to create the genomics in oncology chapter in its committees, as other oncology societies have done in other countries, in an attempt to improve the understanding of the technology available to the clinician today, how it can be used and how to interpret the data obtained.

The following recommendations are based on the best current evidence, divided by *subspecialty*. Groups of specialists in each of the major areas of oncology included oncologists from different treatment centers for cancer patients, both public and private, from all geographic regions of Brazil. Each group was asked to discuss only methods available in the country and that have a real clinical practical use, according to treatments approved by ANVISA or available in open clinical trials in Brazil. The participants of each group met to answer the following

questions regarding the use of genomic panels in their respective areas:

When should a somatic panel test be requested and for which patients? When is the best time to request?

What should be assessed in a somatic mutation panel (which genes should be included and what alterations are expected from each gene)?

Which platforms or tests are the most appropriate?

The answers to these questions were organized in the form of topics, with a subsequent discussion session, to put the data into context and discuss briefly their clinical application.

It is important to comment that when the evidence of a test is evaluated and the recommendation is graded considering the quality of the studies, there is a risk of bias inherent to the molecular testing, including the consistency of the results, its precision, and applicability. For a given test to have a strong recommendation, it is strictly necessary that the test has published data and test validation.

Next-generation sequencing panels (NGS) can identify a specific actionable mutation, but with fair outcomes. Thus, although the test may be sensitive, its applicability has less than ideal results. The degree of recommendation depended on the perception of a panel of specialists and the consensus between them. Thus, a moderate or weak recommendation does not indicate that this is a bad test, but that there was no consensus as to the benefit of its use; this certainly varies depending on the environment in which it is performed. A panel of NGS may be strongly recommended in a center with dozens of phase I studies, but if there are scarce treatments and the absence of clinical trials with new therapies, the degree of recommendation will be weak.

## 2. EVOLUTION OF COMMERCIAL PANELS

In the last few decades, the identification of targeted therapies has paved the way for precision oncology. In parallel, the rapid development of modern sequencing technology has enabled the expansion of the molecular characterization of cancer and sped up the discovery of actionable mutational processes, in addition to the development of targeted therapies. First introduced in 1977<sup>1</sup>, the Sanger sequencing represented a major advance in determining the sequence of nucleotides in the DNA molecule. The Sanger sequencing allowed important discoveries, such as the oncogenic mutations of RAS in 1982<sup>2</sup> and EGFR in 2004<sup>3</sup>. Likewise, the Human Genome Project<sup>4</sup> was entirely carried out using this platform. However, large-scale projects, such as TCGA (The Cancer Genome Atlas), have used new methods of massive parallel sequencing, which have become known as NGS<sup>5</sup>.

NGS methods offer a wide range of possibilities to characterize the cancer genome. First, the NGS allows

the evaluation of hundreds or thousands of genes simultaneously, covering the entire genome or transcriptome within a few days<sup>6</sup>. In addition, the NGS is more sensitive and comprehensive than the Sanger method, as it assesses not only the changes in single nucleotides but also the variation in the number of copies and the multiple structural changes, such as insertions, deletions, and rearrangements, which commonly occur in the cancer genome<sup>6</sup>.

NGS methods have been progressively customized to reduce costs, to prioritize genes associated with cancer and actionable mutations, to be applicable in *formalin-fixed paraffin-embedded tissue samples*; and to provide results on time to become clinically useful<sup>7</sup>. Targeted gene sequencing panels were first introduced in research centers to accelerate precision oncology and patient inclusion directed by biomarkers in phase I and II trials<sup>7-9</sup>. However, commercial panels have emerged as an opportunity for patients and doctors to individualize the use of targeted therapies in clinical practice<sup>10</sup>. Meanwhile, international consensus has been developed to set the recommendations for the use of gene sequencing panels in different scenarios<sup>11</sup>, which further increased the enthusiasm for their use.

Commercially available NGS panels can be classified into two major strategies defined by the target enrichment method: hybridization capture or amplification<sup>12,13</sup>. Target enrichment is the core step in the NGS, as the genes of interest are isolated from the rest of the genome and amplified. This step generates a DNA library, which contains the target regions that will be sequenced and analyzed.

The amplification-based NGS was quickly implemented in clinical practice because it is a simpler process and it has lower cost, shorter delivery time, and its analysis is objective<sup>14</sup>. In addition, local laboratories can adapt in-house panels to cover the gene regions of interest. As a limitation, amplification-based NGS is best suited to cover only DNA hotspot regions and often only to a few dozen genes. On the other hand, hybridization-based NGS (also known as comprehensive genomic profiling) is more easily scalable, it has a high throughput, and represents a more comprehensive strategy, as it allows access to hundreds of genes simultaneously<sup>15-17</sup>. This approach may be more accurate to look for all forms of genomic changes and it includes analyzes such as tumor mutational load and determine microsatellite instability. Other differences between the two NGS techniques include the sequencing process and the bioinformatics algorithm. Such particularities have been reviewed in other publications<sup>12</sup> and are beyond the scope of this article.

A crucial step in the implementation of somatic mutation panels is the expertise in molecular pathology and bioinformatics techniques, which must be accompanied by adequate validation for each step of the process<sup>12</sup>. There are several guidelines on this

topic that must be carefully followed to ensure optimal performance and accuracy<sup>18</sup>.

Given the complexity of the NGS panels, the complete process must be validated, from the extraction of the nucleic acid from the biological material to its final analysis. It is important that the validation process includes the evaluation of the quality of the clinical samples in the paraffin blocks; demonstrate the ability to detect different types of genetic changes; define metrics for routine testing and for supplementary testing in genomic regions that are not reliably sequenced. Common actionable variants must be specifically assessed for accuracy and reproducibility. Orthogonal methods must be applied to confirm the results of the validation process. It is noteworthy that changes in the design of the panels require revalidation before the implementation of a new panel in the clinical practice.

The laboratories must inform whether the NGS panels developed in-house have been properly validated, as well as the possible limitations detected during the process. In addition, the proficiency of the test and the laboratory must be certified to ensure that the NGS processes are followed. Examples of internationally recognized proficiency testing programs are the College of American Pathologists (CAP), Clinical Laboratory Improvements Amendments (CLIA), and United Kingdom National External Quality Assessment (UK NEQAS)<sup>19</sup>. Such efforts to confirm standard testing and analysis is also useful to educate laboratories in the proper reporting of clinically relevant variants<sup>20</sup>.

The USA Food and Drug Administration also provides specific mechanisms for the regulatory approval of NGS for the evaluation of genomic profiles in tumors and it has defined levels of evidence that support the actionability and clinical utility of NGS tests. These programs are crucial to ensure high-quality testing and to support national coverage policies or coverage by health insurance companies.

### 3. CLINICAL APPLICATION FOR THE DIFFERENT TYPES OF CANCER TYPE

#### Thoracic Cancer

When should a somatic panel test be requested and for which patients? When is the best time to request?

1. All patients with non-small cell lung cancer (NSCLC), with any non-squamous histological component; stage IV or recurrent tumor; and candidates for systemic palliative treatment should be tested at the time of diagnosis (type of recommendation: evidence-based; strength of recommendation: strong);
2. Consider testing patients with stage II or III NSCLC treated with curative intent to guide adjuvant treatment (in the case of *EGFR* mutations), and/or to manage a possible future recurrence (type of recommendation: evidence-based; strength of recommendation: moderate);
3. Consider testing patients with pure squamous histology, with little or no exposure to tobacco (type of

recommendation: formal consensus; strength of recommendation: weak).

What should be assessed in a somatic mutation panel (which genes should be included and what alterations are expected from each gene)?

4. As a minimum, somatic mutations in the *EGFR*, *ALK*, *ROS1*, *BRAF*, and *NTRK1-3* genes should be evaluated (type of recommendation: evidence-based; strength of recommendation: strong);

5. In addition to the minimum panel, consider an assessment of somatic alterations in the following genes: *ERBB2*, *MET*, *RET* and *KRAS* (type of recommendation: formal consensus; strength of the recommendation: moderate).

Which platforms or tests are the most appropriate?

6. Prefer the use of sequencing panels containing multiple genes rather than evaluating individual genes sequentially (type of recommendation: formal consensus; strength of recommendation: moderate);

7. Consider the assessment of somatic changes in circulating tumor DNA (liquid biopsy for genotyping), particularly when there are difficulties in obtaining tissue material. If somatic changes are not found in circulating tumor DNA, molecular evaluation in tissue material is recommended (type of recommendation: formal consensus; strength of recommendation: moderate);

8. Alternative methods to gene sequencing can be used for molecular evaluation of lung cancer, such as:

a. Immunohistochemistry (IHC) positive for ALK with antibody D5F3 or 5A4 can be used as a selection criterion for ALK inhibitor therapy. It is considered the preferred method. FISH positive for ALK can be used as a selection criterion for ALK inhibitor therapy (type of recommendation: based on evidence; strength of recommendation: strong);

b. IHC positive for ROS with the D4D6 antibody must be confirmed with FISH and/or NGS before the use of ROS inhibitors. Positive FISH for ROS1 is considered a selection criterion for treatment with ROS inhibitors (type of recommendation: evidence-based; strength of recommendation: strong);

c. IHC for TRK positive with the Pan-TRK antibody: TRKA, TRKB, and TRKC, or VENTANA pan-TRK EPR17341. It can be used as a selection criterion for TRK inhibitor therapy, however, confirmation by FISH or NGS is preferable (type of recommendation: formal consensus; strength of the recommendation: moderate);

d. Lung cancer data are insufficient to recommend IHC for BRAF V600E as a selection criterion for treating patients with BRAF inhibitors (type of recommendation: formal consensus; strength of recommendation: moderate);

e. IHC for EGFR should not be used to select patients who are candidates for treatment with EGFR inhibitors (type of recommendation: based on evidence; strength of recommendation: strong).

## DISCUSSION

The use of targeted palliative therapy in patients selected according to the molecular profile increases progression-free survival and overall survival when compared to cytotoxic chemotherapy<sup>21</sup>. Controlled studies with targeted therapy included only patients with adenocarcinoma, a predominance of adenocarcinoma, or mixed histology with an adenocarcinoma component. Patients without a definitive diagnosis of adenocarcinoma (for example, carcinoma not otherwise specified, NOS) may have driver mutations (particularly in inadequate/unrepresentative biopsy samples) and should also be treated with targeted therapy in this situation. Driver mutations are also found in patients with squamous histology, with little or no exposure to tobacco (1 to 10 pack-years)<sup>22</sup>.

The decision to test patients with disease at an earlier stage should consider the cost of obtaining such tests in individuals who can be cured (and for which the results will be of little or no use) versus the advantage of knowing the molecular profile earlier if there is cancer recurrence<sup>11,22</sup>. Driver mutations of clinical relevance, in general, are present since the initial diagnosis in patients with less advanced stages and they remain stable throughout the natural history of the disease<sup>23</sup>. The targeted therapy as adjuvant or non-palliative therapy is being studied<sup>24</sup>. The use of adjuvant osimertinib is beginning to be considered as it was associated with increased disease-free survival versus placebo in patients with resected stage II-III NSCLC<sup>25</sup>.

The minimum molecular panel should include assessment of *EGFR*, *ALK*, *ROS*, *BRAF*, and *NTRK1-3*. This panel reflects the approval of targeted therapies in Brazil and should be expanded as new drugs demonstrate clinical benefits (Table 1). *EGFR* inhibitors are recommended as first-line treatment of patients with *EGFR* sensitivity mutations due to increased progression-free survival, response rate, and quality of life when compared to chemotherapy<sup>26</sup>. Patients with *EGFR* mutations treated with first or second-generation inhibitors, often develop the secondary T790M resistance mutation at the time of progression. The use of osimertinib in these patients is considered the gold standard of treatment, according to the results of a phase 3 study, which makes the assessment of T790M mutation mandatory in this scenario<sup>27</sup>. The presence of *ALK* gene rearrangements is associated with high sensitivity to ALK-TKIs (crizotinib, ceritinib, alectinib, brigatinib and lorlatinib)<sup>28</sup>.

Due to the high degree of homology in the tyrosine kinase domains of ALK and ROS, crizotinib showed a high response rate and disease control in patients with *ROS1* fusion, being the drug of choice in the first-line treatment of these patients<sup>29</sup>. The presence of a *BRAF* V600E mutation does not occur with other molecular changes, except for the *KRAS* mutation, which can coexist with other types of *BRAF* mutations<sup>30</sup>. The combination of dabrafenib and trametinib for the treatment of patients with the *BRAF* V600E mu-

tation is approved in Brazil and is based on phase 2 studies<sup>31</sup>. Fusion in one of the three TRK receptors confers sensitivity to larotrectinib in lung cancer and other tumors TRK fusion-positive. This is the first approved agnostic therapy in Brazil<sup>32</sup>.

The expanded panel testing can detect genetic changes that have drugs approved for other types of tumors but with activity in lung cancer (for example, HER2 blockade with trastuzumab, afatinib, or T-DM1 in the presence of *ERBB2* mutations or HER2 amplification)<sup>33</sup>. It also allows repositioning of approved drugs to other scenarios (for example, use of crizotinib for tumors with *MET* amplification or exon 14 skipping mutations<sup>34</sup> and the use of drugs already approved in other countries (for example, capmatinib for lung cancer exon 14 skipping mutation of *MET*<sup>35</sup> and selpercatinib for lung cancer with *RET* translocation)<sup>36</sup> and inclusion of patients in studies with new drugs<sup>37</sup>.

Often, a hotspot panel is used to test patients for the most common genomic alterations<sup>38</sup>. Sequences of single-gene testing can also be used without a hotspot panel, testing the most frequent alterations initially, and after that the rarest ones. In both cases, running a sequence of single-gene testing is time-consuming and may require a relatively large amount of tissue sample<sup>39</sup>.

Next-generation sequencing (NGS) has emerged as a reliable method to test several alterations simultaneously using a single tissue sample<sup>40</sup>. Computerized models demonstrated that NGS was associated with the same duration to test result (when compared to the hotspot panel) or less (when compared to sequential or exclusion tests) at reduced costs<sup>41</sup>. The use of NGS resulted in the identification of almost 40% more patients with genomic alterations, some with targeted therapies not yet approved by regulatory agencies. These patients could be candidates for clinical trials<sup>42</sup>.

Liquid biopsy in plasma may overcome some limitations of solid tissue biopsy<sup>43</sup>. The circulating tumor DNA may reflect the genetic profile of the tumor, therefore, the possibility to characterize it may have prognostic and therapeutic value<sup>44,45</sup>. Nevertheless, liquid biopsy can fail to detect low levels of circulating tumor DNA (either due to low tumor load or low DNA released by the tumor), which can lead to false-negative results. The agreement of the liquid biopsy with biopsy of the tumor tissue depends on the percentage of tissue changes found in the blood<sup>46,47</sup>, the size of the tumor tissue sample, the timing of the sample, in addition to other factors, such as tumor heterogeneity, treatment interval, and method used<sup>45,48,49</sup>.

In addition to gene sequencing, the molecular profile of lung cancer can be determined by alternative methods, including IHC and FISH for specific genes. The D5F3 and 5A4 antibodies to ALK have a sensitivity of 96% and specificity of 100% for the diagnosis of NSCLC with *ALK* translocation<sup>50</sup>, and they are considered as standard testing for patients' selection ac-

**Table 1.** Main somatic alteration in non-small cell lung cancer and non-squamous lung cancer

Gene / Alteration	Alteration	Test	Comments
<i>EGFR</i> (10-25%)	Mutations in exons 18-21. The most frequent (90% of cases) are exon 19 deletions and exon 21 L858R substitution mutation.	NGS RT-PCR dd-PCR	Unusual mutations in exon 20 are often associated with resistance. <i>EGFR</i> T790M mutation assessment is necessary if there are indication of first or second-generation tyrosine kinase inhibitors
<i>ALK</i> (2-5%)	Gene translocations (rearrangements)	NGS Alternative methods: IHC with D5F3 or 5A4 antibodies or FISH	The main partner is the <i>EML4</i> gene, which determines the <i>EML4-ALK</i> fusion oncogene
<i>ROS1</i> (1-2%)	Gene translocations (rearrangements)	NGS Alternative methods: FISH / IHC positive with D4D6 antibody must be confirmed with FISH and/or NGS	
<i>BRAF</i> (2%)	V600E point mutation	NGS	Other mutations in the <i>BRAF</i> gene are not considered drivers
<i>NTRK 1,2 e 3</i> (< 1%)	Gene translocations (rearrangements)	NGS Alternative methods: FISH or IHC with antibody (pan-TRK: TRKA, TRKB, and TRKC, or VENTANA pan-TRK EPR17341)	
<i>HER2</i> (1-3%)	Two deregulation mechanisms can occur: Gene amplification/overexpression or exon 20 insertion mutation	NGS	Exon 20 point mutation can rarely occur
<i>MET</i> (3%)	Exon 14 skipping mutation	NGS	Gene amplification is involved in resistance to <i>EGFR</i> tyrosine kinase inhibitors
<i>RET</i> (1-2%)	Gene translocations (rearrangements)	NGS	
<i>KRAS</i> (20%)	Point mutation	NGS	The actionability is related to the finding of <i>KRAS</i> G12C alteration

According to ASCO, ESMO, and NCCN guidelines<sup>11,24,46</sup>. FISH for *ALK* is also an acceptable method for detecting *ALK* translocation, but it has disadvantages in comparison with NGS, such as the impossibility of defining the translocation partner and less sensitivity<sup>50</sup>. IHC for ROS may be used as a screening test for tumors with *ROS1* translocation. The D4D6 antibody to ROS can label non-neoplastic cells and, therefore, positive results must be confirmed by FISH or NGS<sup>51</sup>. The FISH for *ROS1* has a sensitivity of 100% and a specificity of 92%<sup>51</sup>.

Several alternative methodologies can be used to detect *NTRK* fusions, including FISH and IHQ. The FISH may

need up to three probes for a complete analysis<sup>11</sup>. The anti-*BRAF* V600E monoclonal antibody is commercially available<sup>11</sup>, however, it is necessary validation for the detection of *BRAF* mutations in lung cancer.

Head and Neck Cancer

When should a somatic panel test be requested and for which patients? When is the best time to request?

What should be assessed in a somatic mutation panel (which genes should be included and what alterations are expected from each gene)?

Which platforms or tests are the most appropriate?

Squamous cell carcinoma of the upper aerodigestive tract

1. Routine somatic panel is not indicated. IHC to assess overexpression of p16 (determine whether HPV positive or negative) in the oropharynx is mandatory for staging and prognostic assessment (type of recommendation: evidence-based; strength of evidence: strong). In metastatic disease, PD-L1 assessment using IHC (CPS) is necessary to define treatment (type of recommendation: based on evidence; strength of evidence: strong).

Nasopharyngeal carcinoma

2. Routine somatic panel is not indicated. IHC or in situ hybridization (preferred) may be performed in the tumor sample to assess etiological correlation with the Epstein-Barr virus (EBV) (type of recommendation: evidence-based; strength of evidence: strong).

Salivary gland carcinomas

3. In metastatic disease: 1) IHC or in situ hybridization to assess HER2 overexpression (type of recommendation: evidence-based; strength of evidence: moderate); 2) IHC to assess androgen receptor expression (type of recommendation: evidence-based; strength of evidence: moderate); 3) Assess *NTRK1-3* fusion, initially by IHC-pan-TRK and, if positive, confirmation with NGS (type of recommendation: evidence-based; strength of evidence: moderate).

Thyroid cancer

Differentiated thyroid carcinoma

4. Routine somatic panel is not recommended. In metastatic disease, it is recommended: 1) investigation of *NTRK1-3* fusion, either by initial IHC pan-TRK screening (EPR 17341 Abcam or Roche / Ventana) and, if positive, confirmation with NGS, or direct testing by NGS (type of recommendation: evidence-based; strength of evidence: moderate); 2) NGS for *RET* fusion (type of recommendation: evidence-based; strength of evidence: moderate).

Medullary thyroid carcinoma

5. Routine somatic panel is not recommended. It is recommended in advanced or metastatic disease: NGS to assess *RET* mutations in tumor tissue and to assess germline mutation in suspected cases of multiple endocrine neoplasia type 2 (recommendation type: evidence-based; strength of evidence: strong).

Anaplastic thyroid carcinoma

6. Routine somatic panel is not recommended. It is recommended in locally advanced or metastatic disease: 1) RT-PCR (real-time polymerase chain reaction) or NGS to assess BRAF V600E mutation (type of recommendation: evidence-based; strength of evidence: moderate); 2) IHC for pan-TRK as a screening test and, if positive, confirmation with NGS, preferably RNA (type of recommendation: evidence-based; strength of evidence: moderate). Routine assessment of PD-L1 or TMB (tumor mutational burden) is not recommended.

## DISCUSSION

Head and neck carcinoma is a heterogeneous group of epithelial tumors that initiate in the oral cavity, larynx, pharynx (upper aerodigestive pathways), paranasal sinuses, salivary glands, and thyroid.

Head and neck squamous cell carcinoma (HNSCC)

HNSCC represents 90% of head and neck tumors. In addition to clinical, phenotypic, and etiologic heterogeneity, these tumors have high molecular heterogeneity<sup>52</sup>. There are currently no predictive genetic markers of response useful to determine the treatment. Therefore, routine panels are not recommended to assess somatic mutations for HNSCC.

P16 protein overexpression by IHC in the sample of oropharyngeal tumors (positivity higher than 70% of the sample) is a marker of the presence of the human papillomavirus (HPV) and it is mandatory for staging and prognostic evaluation<sup>53</sup>. For metastatic HNSCC in cervical lymph nodes of unknown primary site, the presence of p16 (by IHC) should be assessed in the tumor tissue and, if negative, assess the presence of the EBV virus by IHC or in situ hybridization, preferably<sup>53</sup>. In metastatic disease, it is required IHC assessment of PD-L1 expression (22C3 pharmDx), characterized by CPS (combined proportional score).

A phase III study (KEYNOTE 048) compared pembrolizumab alone or in combination with cisplatin and 5-fluorouracil versus the EXTREME regimen (cisplatin, 5-fluorouracil, and cetuximab)<sup>54</sup>. This study showed higher overall survival with isolated pembrolizumab in overall survival in patients with HNSCC with CPS $\geq$ 1% (12.3 versus 10.3 months, HR = 0.74) and with pembrolizumab associated with cisplatin and 5-fluorouracil, regardless of CPS (13.0 versus 10.7 months, HR = 0.72), compared to the EXTREME arm. Patients with CPS<1% did not show any benefit in comparison to the use of immunotherapy in first line therapy<sup>55</sup>.

Salivary glands cancer

In salivary gland cancer, the assessment of therapeutic targets is recommended in some patients. In the head and neck neoplasms, this would be the scenario in which somatic mutation panels would be closer to use in clinical practice.

*NTRK1-3* fusion is described in 90-100% of secretory salivary gland carcinomas (also known as MASC)<sup>56,57</sup>, particularly the *ETV6-NTRK3*<sup>58,59</sup> fusion. They may also be present in 2-15% of papillary thyroid carcinomas<sup>60-62</sup> and in less than 1% in the other head and neck tumors<sup>63,64</sup>.

IHC screening using pan-TRK antibody (EPR 17341 Abcam or Roche / Ventana)<sup>65</sup> is recommended, followed by NGS for confirmation, with *NTRK1*, *NTRK2*, and *NTRK3* fusion assessment, preferably based on RNA<sup>66</sup>. If there is a histological diagnosis of MASC, NGS may be performed as the initial test. For a metastatic disease with an indication for systemic

treatment and the presence of *NTRK* fusion, first-line treatment with larotrectinib<sup>32</sup> is recommended. Entrectinib is another option in this scenario<sup>67</sup>, although it is not available in Brazil.

HER2 overexpression is present in up to 30% of mucoepidermoid carcinomas or adenocarcinomas not otherwise specified (NOS)<sup>68,69</sup>, and in up to 40% of salivary ductal carcinomas<sup>68,70-75</sup>. Although there is no consensus, most studies evaluate the HER2 expression in breast cancer<sup>76,77</sup>, being considered positive IHC 3+ or 2+ and FISH with ratio HER2/CEP17  $\geq 2$ <sup>68,78</sup>. In metastatic disease, treatment with a combination of chemotherapy (taxanes with or without platinum) and trastuzumab is supported by a case series<sup>79,80</sup> and a phase II study<sup>78</sup>. There are also data for double blockade with trastuzumab and pertuzumab<sup>81,82</sup>. Trastuzumab-emtansin (T-DM1) can be used in the second line<sup>83,84</sup> and there are some data on the efficacy of trastuzumab-deruxtecan<sup>85</sup>. The use of anti-HER2 therapy as adjuvant treatment was evaluated only in retrospective studies<sup>79,86</sup>.

IHC androgen receptor (AR) expression, characterized as nuclear, strong, and diffuse (>70%), is observed in most salivary ductal carcinomas<sup>87-91</sup> and adenocarcinomas NOS. The use of combined hormonal blockade, with a GnRH agonist and bicalutamide, has shown efficacy in metastatic disease with AR expression in retrospective studies<sup>92-94</sup> and a prospective phase II study<sup>95</sup>. A randomized phase 2 study comparing hormonal blockade and chemotherapy in this scenario is ongoing<sup>96</sup>. The use in the adjuvant setting was evaluated only retrospectively<sup>97</sup>.

#### Thyroid cancer

In metastatic radiiodine-refractory differentiated thyroid carcinoma (DTC), the presence of *NTRK* fusions is estimated in up to 12% of patients<sup>64</sup>. It is recommended to screen with IHC composed of pan-TRK antibody (EPR 17341 Abcam or Roche / Ventana)<sup>65</sup>, followed by NGS confirmation, with *NTRK1*, *NTRK2*, and *NTRK3* fusion, preferably based on RNA<sup>66</sup>. For metastatic patients with indication for systemic treatment and presence of *NTRK1-3* fusion, it is recommended first-line treatment with larotrectinib - already approved in Brazil. This approval was based on a pooled analysis of three studies with seven individuals with advanced thyroid cancer. There was a 100% response to larotrectinib in this population<sup>32</sup>. *RET* rearrangement was found in approximately 20% of these patients<sup>98</sup>.

The FDA recently approved selpercatinib for first-line treatment of metastatic radiiodine-refractory DTC with *RET* fusions. This drug showed very encouraging results in a phase 1/2 study. Particularly in DTC, the response rate was 100% when used in the first-line and 79% in other treatment lines<sup>36</sup>. It is recommended to assess *RET* fusion by NGS<sup>98</sup>.

In medullary thyroid carcinoma (MTC), *RET* activation is proven to be one of the main mechanisms of oncogenesis. In patients with sporadic MTC, *RET* somatic mutations are found in approximately 40-60% of patients<sup>99</sup>. The results of the LIBRETTO-001 study with

selpercatinib demonstrated a response rate of 73% in the first-line, particularly in MTC, and 69% in previously treated patients<sup>36</sup>. NGS is indicated to assess *RET* mutations in tumor tissue<sup>98</sup>.

In anaplastic thyroid carcinoma (ATC), the *BRAF* V600E mutation is found in 20% to 50% of patients<sup>100</sup>. The combination of dabrafenib and trametinib is already approved for the treatment of patients with locally advanced or metastatic ATC with *BRAF* V600E mutation without effective locoregional treatment options. A 69% response rate was demonstrated with the combination, being the preferred strategy in this condition<sup>101</sup>. For these patients, it is recommended the assessment of this mutation by RT-PCR or NGS<sup>102</sup>. *NTRK* fusions are also relevant in ATC.

As previously mentioned, larotrectinib shows encouraging results in this population and the screening of these patients should be performed with IHC composed of pan-TRK antibody (EPR 17341 Abcam or Roche / Ventana) 65, followed by NGS for confirmation<sup>66</sup>.

#### Gastrointestinal Cancer

When should a somatic panel test be requested and for which patients? When is the best time to request?

1. In the diagnosis of unresectable metastatic or locally advanced disease in esophageal, gastric, pancreatic, biliary, intestinal (small intestine and colorectal) adenocarcinomas (type of recommendation: evidence-based; strength of recommendation: strong);
2. In the diagnosis of the localized esophageal and gastric adenocarcinoma stage II and III (type of recommendation: evidence-based; strength of the recommendation: strong);
3. In the diagnosis of the localized colorectal adenocarcinoma stage II (type of recommendation: evidence-based; strength of the recommendation: strong).

What should be assessed in a somatic mutation panel (which genes should be included and what alterations are expected from each gene)?

4. In metastatic or unresectable adenocarcinomas or poorly differentiated carcinomas:
  - a) Microsatellite instability (MSI) assessment (type of recommendation: evidence-based; strength of recommendation: strong).
  - b) *NTRK1-3* fusion assessment (neurotrophic tyrosine receptor kinase) fusion research (recommendation type: evidence-based; recommendation strength: strong).
5. Esophageal and gastric adenocarcinoma:
  - a) unresectable metastatic/locally advanced disease: assessment of HER2 overexpression or *ERBB2* amplification (Human epidermal growth factor receptor; overexpression or amplification (type of recommendation: evidence-based; strength of recommendation: strong);
  - b) localized disease: MSI (microsatellite instability) assessment (type of recommendation: evidence-based; strength of recommendation: strong).



## 6. Biliary tract cancer:

a) unresectable metastatic / locally advanced disease: *FGFR2* (fibroblast growth factor receptor 2) fusion or rearrangement assessment (type of recommendation: formal consensus; strength of recommendation: strong); *IDH1* mutation (isocitrate-dehydrogenase) (type of recommendation: evidence-based; strength of recommendation: moderate); *BRAFV600E* mutation (type of recommendation: formal consensus; strength of the recommendation: strong); *ERBB2* amplification (type of recommendation: formal consensus; strength of the recommendation: weak).

## 7. Colorectal adenocarcinoma:

a) metastatic disease (stage IV): *KRAS* and *NRAS* mutation assessment (exon 2: codons 12, 13; exon 3: codons 59 to 61; exon 4: codons 117 and 146) (type of recommendation: evidence-based; recommendation strength: strong); *BRAF V600E* mutation (recommendation type: ev-

idence-based; recommendation strength: strong); *HER2* overexpression or *ERBB2* amplification (type of recommendation: formal consensus; strength of recommendation: moderate); *POLE* (DNA polymerase epsilon) mutation (type of recommendation: formal consensus; strength of the recommendation: weak).

b) stage II disease: MSI assessment (type of recommendation: evidence-based; strength of recommendation: strong)

## 8. Neuroendocrine tumors:

a) metastatic disease, G3 / Neuroendocrine carcinoma (NEC): *BRAF V600E* mutation assessment (type of recommendation: formal consensus; strength of the recommendation: weak).

Which platforms or tests are the most appropriate?

Are there alternative tests in clinical practice (e.g. single-gene sequencing, FISH, IHC, etc.)? If there

**Table 2.** Main somatic alterations in head and neck cancer.

Types of cancer	Gene / Alteration	Test	Comments
Head and neck (squamous cell carcinoma)	There is no gene with validated actionable alteration	IHC for p16 overexpression (determining whether HPV positive or negative) in the oropharynx is mandatory for staging and prognostic evaluation (positive in 70% of patients) IHC for PD-L1 (CPS) for the definition of treatment in the metastatic disease	There is no recommendation for somatic sequencing test
Nasopharyngeal carcinoma	There is no gene with validated actionable alteration	IHC or in situ hybridization (preferred) in the tumor tissue sample to assess etiological correlation with the Epstein-Barr virus	There is no recommendation for somatic sequencing test
Salivary gland carcinoma	<i>NTRK 1-3</i>	IHC for HER-2 and androgen receptor (AR) expression IHC pan-TRK for assessment of <i>NTRK 1-3</i> fusion; if positive, confirm with NGS	There is no recommendation for somatic sequencing testing in the absence of positive screening for pan-TRK
Differentiated thyroid carcinoma (papillary, follicular)	<i>NTRK 1-3</i> <i>RET</i>	IHC: pan-TRK (EPR 17341 Abcam or Roche / Ventana) for screening and, if positive, confirmation with NGS (positive in 12% of patients) NGS to assess <i>RET</i> fusion in tumor tissue (20%)	
Medullary thyroid carcinoma	<i>NTRK 1-3</i>	NGS to assess <i>RET</i> fusion in tumor tissue (40-60%)	Germline mutation assessment is recommended for suspected cases of multiple endocrine neoplasia type 2
Anaplastic thyroid carcinoma	<i>NTRK 1-3</i> <i>BRAF</i>	NGS for <i>BRAF V600E</i> mutation assessment (20-50%) ICQ pan-TRK as a screening test and, if positive, it is necessary confirmation with NGS.	

are alternative tests, whenever possible, determine whether the panel is the preferred one.

9. **HER2 / ERBB2:** IHC complemented by in situ hybridization test (ISH), such as FISH, if the initial results are inconclusive. In IHC, the expression of the antibody is graded in a score of 0, 1, 2, or 3 crosses. Score 3+ indicates overexpression; Score 2+ is doubtful and it is recommended to do the FISH. The amplification or mutations evidenced by NGS panels should not be used as criteria for treatment in the absence of IH positivity.

10. **MSI/MMR:** IHC, PCR, or NGS. IHC is the most available and least expensive test, however, as it is an indirect method, it has a greater probability of errors, and should be confirmed by a direct method when positive. IHC includes assessment of the expression of MLH1, MSH2, MSH6, and PMS2 repair proteins. The absence of expression means the presence of MSI. IHC alone does not detect 10% of patients with MSI, so, if available, negative tests should be repeated with molecular analysis. The most widely used validated panel in the world for the assessment of microsatellite instability includes five monomorphic markers (pentaplex panel). The presence of two unstable markers indicates a status of high instability. The presence of one or no unstable marker characterizes a state of low instability or stable microsatellites;

11. **NTRK:** NGS. IHC maybe be used for screening, since it is rapid and less expensive. However, positive cases must be evaluated with sequencing to confirm the fusion;

12. **KRAS/NRAS:** The ideal test is the sequencing of the gene by real-time PCR, which may or may not be part of a panel with other genes. In the absence of the method, it is common and reliable to assess hotspots by simple sequencing, or even by using conventional PCR with restriction enzymes;

13. **BRAF V600E:** The ideal test is the sequencing of the gene by real-time PCR, whether or not it may be part of a panel with other genes. In the absence of the method, it is common and reliable to assess the most common alterations in hotspots by simple sequencing, or even by using conventional PCR with restriction enzymes;

14. **FGFR2:** Sequencing of the gene by real-time PCR, which may or may not be part of a panel with other genes;

15. **IDH1:** Sequencing of the gene by real-time PCR or by NGS;

16. **POLE:** Sequencing of the gene by real-time PCR or by NGS. They are usually part of the NGS panel, and the high number of mutations found in tumor cells, called the ultramutated genotype, is characteristic.

Obs. Due to the cost of each analysis and the time spent, panels by NGS tend to quickly replace the above technologies, addressing all of these genes with deep sequencing simultaneously.

## DISCUSSION

### Agnostic alterations

Microsatellite instability (MSI) - Microsatellites are simple sequences (repeats) of nucleotides that occur throughout the genome. Its instability is a marker of mismatch repair (MMR) deficiency, a system composed of four enzymes encoded by the *MLH1*, *MSH2*, *MSH6*, and *PMS2* genes, whose dysfunction can be germinative (Lynch syndrome) or somatic, more often associated with epigenetics changes (methylation of the promoter region of the gene). The detection of this alteration in somatic panels by different methodologies (section 1), in addition to screening individuals and families with Lynch syndrome, is associated with a potential response to immunotherapy with immune checkpoint inhibitors (ICI) in several histologies<sup>103-105</sup>. This approach was the first agnostic cancer therapy approved in many countries after failure in at least one treatment line.

At the ASCO 2020 plenary sessions, the KEYNOTE-177 study showed benefit in overall survival (OS), progression-free survival (PFS), and response rate in favor of pembrolizumab when compared to chemotherapy chosen by the investigator and monoclonal antibody in the first line of metastatic colorectal cancer<sup>106</sup>. It is also important to cite that the presence of *POLE* or *POLD1* mutations are also associated with a better prognosis in the initial disease and benefit with immunotherapy<sup>107</sup>. In stage II colorectal cancer, the presence of MSI-H was associated with a better prognosis, so that adjuvant chemotherapy is considered ineffective and is not recommended in this scenario.<sup>108</sup>

In esophageal and gastric adenocarcinoma, the presence of MSI-H was associated with lack of efficacy of chemotherapy for localized disease in post-hoc analysis of phase III clinical trials<sup>109,110</sup>, and its use is considered controversial, particularly in the perioperative scenario.

**NTRK fusions and rearrangements** - The *NTRK* genes encode the tropomyosin receptor kinase (TRK). Fusion of these genes leads to overexpression of fusion proteins with TRK, which results in persistent signaling in different tumors<sup>111</sup>. The activity of NTRK tyrosine kinase inhibitors (TKI) occurs in several histologies<sup>32</sup>. The use of TRK inhibitor agents should be considered in therapies after the first-line in these patients. Larotrectinib is the drug currently available in Brazil.

Note: In a cohort of 2,314 patients with metastatic colorectal cancer, *NTRK* alterations were found in patients without mutations, that is, *KRAS*, *NRAS*, and *BRAF* wild-type. Seven of the eight *NTRK* fusions in this analysis occurred in MSI-H patients<sup>112</sup>. If confirmed, this finding may restrict the metastatic colorectal cancer population for whom the test would be recommended.

### Esophageal and gastric cancer

**HER2** - The HER2 protein is a transmembrane receptor tyrosine kinase, a member of the epidermal growth factor (EGFR) receptor family and responsi-

ble for the regulation of cell proliferation, differentiation, and survival<sup>113</sup>. About 7-20% of esophageal and gastric adenocarcinomas are HER2 positive, that is, they have IHC expression for HER2 3+ or 2+ with positive FISH (fluorescence in situ hybridization), or CISH (chromogenic in situ hybridization)<sup>114</sup>. The positivity rates are similar between European and Asian patients (23.6% vs. 23.9%), but they are higher in the intestinal type than in the diffuse type (31.8% vs. 6.1%), and in esophagogastric junction adenocarcinoma than in gastric tumors (32.2% vs. 21.4%)<sup>115</sup>.

The ToGA study evaluated a humanized monoclonal antibody against HER2 – the trastuzumab – demonstrating better PFS and OS with the trastuzumab and chemotherapy in patients with locally advanced, unresectable, or metastatic gastric or esophageal cancer, HER2 positive, compared to chemotherapy alone<sup>115</sup>. The DESTINY-Gastric01 study was an open-label, phase 2, randomized study that demonstrated that trastuzumab deruxtecan (DS-8201), compared to the treatment chosen by the investigator, increased the response rate and OS in refractory patients in at least two lines of treatment, including trastuzumab<sup>116</sup>.

### Biliary Tract Cancer

**FGFR** - The fibroblast growth factor receptor is a transmembrane receptor with a tyrosine kinase domain, divided into four subtypes (FGFR1-4). Between 6 and 15% of cholangiocarcinomas have some alteration in FGFR gene. Fusion is the most frequent alteration<sup>117</sup>. They are more common in intrahepatic cholangiocarcinomas and, in general, are associated with better prognosis<sup>118</sup>. Pemigatinib is a FGFR1, -2 and, -3 inhibitor drug approved by the FDA in 2020 for use in patients with *FGFR2* fusion. The approval is based on the phase 2 study FIGHT-202 that included 107 patients with *FGFR2* fusion who had failed at least one previous chemotherapy and were treated with pemigatinib. The response rate and the disease control of the disease were 35% and 88%, respectively, with a median PFS of 6.9 months<sup>119</sup>. Mutated patients showed no benefit from using the drug.

**IDH1/2** - *IDH1/2* mutations are found in up to 7% of extrahepatic cholangiocarcinomas, and up to 15% of intrahepatic cholangiocarcinomas. Generally, these mutations are mutually exclusive to *FGFR* alterations. Ivosidenib is a *IDH1* inhibitor used for the treatment of leukemia with *IDH* alteration. The phase III study ClarIDHy randomized 185 patients with chemotherapy failure to receive ivosidenib or placebo. The ivosidenib group had the highest number of patients with stable disease and the highest PFS<sup>121</sup>.

**BRAF** - *BRAF* mutations occur in 1-7% of biliary tract cancer, most in intrahepatic cholangiocarcinomas. The most common alteration is V600E mutation<sup>118</sup>. Particularly in patients with *BRAF* V600E mutation, the dabrafenib-trametinib combination was evaluated in the ROAR study that included in which 35 patients were treated with a response rate of 36% and a PFS of 9.2-month<sup>122</sup>.

**HER2 - *ERBB2*** amplifications occur in 1-3% of intrahepatic cholangiocarcinomas and 10-16% of extrahepatic and gallbladder tumors<sup>118</sup>. There are reports of HER2 overexpression as a mechanism of acquired resistance to FGFR inhibitors. Data on anti-HER2 drugs are limited to a retrospective case series, with response rates of up to 50% for gallbladder tumors treated with trastuzumab, with or without pertuzumab<sup>123</sup>. The largest prospective study on anti-HER2 therapies for biliary tract cancer is MyPathway, in which 11 patients with *ERBB2* amplification or mutation were treated with trastuzumab-pertuzumab, resulting in a response rate of 36% and a duration of response of 4.2 months<sup>124</sup>.

### Colorectal carcinoma

**KRAS, NRAS, and HRAS** - The three human RAS genes (*KRAS*, *NRAS*, and *HRAS*) are frequently altered by somatic mutations in several tumors, including colorectal<sup>125</sup>. RAS is a downstream component of the EGFR pathway. Monoclonal anti-EGFR antibodies act by blocking the signal chain and preventing cell proliferation. However, when there are mutations in the genes that encode RAS, this pathway is constantly activated. *KRAS* is mutated in approximately 40% of colorectal tumors<sup>126,127</sup>, representing an early event in carcinogenesis and it was the first mutation identified as a negative predictive marker concerning to the use of anti-EGFR monoclonal antibodies. *NRAS* mutation, although less frequent (5-10%), is also a negative predictor of response to anti-EGFR. Currently, it is being tested together with *KRAS* and named all-RAS. Cetuximab and panitumumab are monoclonal anti-EGFR antibodies that have shown benefit in patients with wild RAS metastatic colorectal cancer, particularly when the primary tumor is located in the left colon<sup>128-132</sup>.

**BRAF** - the *BRAF* gene codes the protein kinase serine-threonine and acts as a downstream effector of RAS signaling, and is a component of the RAS-RAF-MEK-MAPK pathway<sup>133</sup>. The V600E mutation occurs in 8-10% of metastatic colorectal adenocarcinomas. It is more common in tumors on the right side and it is associated with a worse prognosis<sup>134</sup>. Mutations in the RAS and *BRAF* V600E family genes are mutually exclusive<sup>135</sup>.

Studies have shown that treatment with the triple chemotherapy combination FOLFOXIRI, associated or not with bevacizumab, is superior to double combinations<sup>136</sup>. In patients with *BRAF* V600E mutation and previous treatment, a randomized phase II study showed benefits with an association of a *BRAF* inhibitor (vemurafenib), chemotherapy (irinotecan) and anti-EGFR antibody (cetuximab)<sup>137</sup>. More recently, the BEACON phase III study showed benefit increasing OS, PFS, and response rate with the combination of *BRAF* inhibitor (encorafenib) plus anti-EGFR antibody (cetuximab), with or without MEK inhibitor (binimetinib), in previously treated patients. Subsequently, it was approved by the FDA<sup>138,139</sup>.

The *BRAF* V600E mutation is also a negative predictor of benefits with anti-EGFR therapy, as shown in a

meta-analysis<sup>140</sup>. There are other *BRAF* mutations, not V600E, that do not have the same prognostic value as V600E, resembling wild *BRAF*. These mutations may occur concomitant with mutations in the genes of the RAS family in approximately 30% of the patients<sup>141</sup>.

**HER2** - The *ERBB2* amplification or overexpression is present in 6% of wild RAS metastatic colorectal adenocarcinomas and it is considered a mechanism of resistance to anti-EGFR therapy. In the HERACLES study, 27 patients with wild RAS metastatic colorectal cancer, HER2+, refractory to standard therapies, received trastuzumab and lapatinib, with a response rate of 34%<sup>142</sup>. In the phase IIa MyPathway study, 57 patients with colorectal cancer HER2+ received trastuzumab-pertuzumab with a response rate of 32%<sup>1124</sup>. Recently, the phase II study Destiny-CRC01 included patients with HER2-positive metastatic colorectal cancer (IHC 3+, or IHC 2+/ISH+), previously treated. The study demonstrated the activity of trastuzumab-deruxtecan and with a response rate of 45%, regardless of the previous exposure to anti-HER2. In the sample, 30% had received anti-HER2 therapy<sup>143</sup>.

### Gynecologic Cancer

When should a somatic panel test be requested and for which patients? When is the best time to request?

According to current evidence, there is benefit in performing a somatic panel only in ovarian and endometrial cancers, as detailed below.

### Endometrial cancer

What should be assessed in a somatic mutation panel (which genes should be included and what alterations are expected from each gene)? How? Which platforms or tests are the most appropriate?

1. All patients with endometrial carcinoma (EC), regardless of histology and staging, should be investigated for mismatch repair (MMR) deficiency at the diagnosis, which may be by microsatellite instability research (MSI), next-generation sequencing (NGS), or by assessment of MMR proteins - *MLH1*, *MSH2*, *MSH6*, and *PMS2* - expression by IHC. There is evidence of a high agreement between MSI and IHC for MMR proteins expression in the EC (type of recommendation: evidence-based; strength of the recommendation: strong);
2. At diagnosis, it is advisable to carry out a *POLE* genes mutation assessment (by NGS) and a p53 expression assessment (IHC). It is also advisable to perform L1CAM assessment (IHC) in these patients (type of recommendation: evidence-based; strength of the recommendation: weak);
3. Patients with a diagnosis of serous EC, FIGO III / IV or recurrent disease should be considered for HER2 screening by IHC, followed by FISH in uncertain cases (type of recommendation: evidence-based; strength of recommendation: moderate);
4. It is recommended to assess the presence of hormone receptors, progesterone and estrogen, by IHC in stage III, IV or relapsed EC (endometrioid histology)

(type of recommendation: evidence-based; strength of recommendation: moderate).

## DISCUSSION

Due to the high prevalence of MMR deficiency in EC, the NCCN and the Society of Gynecologic Oncology guidelines recommend its universal assessment at the time of diagnosis, and the assessment of MSI by NGS or assess MMR proteins by IHC<sup>144,145</sup>. Up to 40% of EC may have MSI, but only 3-5% have a germline mutation in the MMR/Lynch Syndrome<sup>146</sup>.

A recent study demonstrated disagreement between the tests (MSI-high in NGS and absence of MMRd in IHC) in 5% of the cases of EC<sup>147</sup>. Patients with discordant tests had a tumor mutation burden (TMB) similar to the concordant ones and they have higher rates of immunotherapy response.

In 2013, with the publication of The Cancer Genome Atlas Research Network (TCGA), it was possible to subdivide epithelial endometrial cancer (endometrioid and serous) into four molecular groups: a) ultra-mutated *POLE*, characterized by a mutation in the *POLE* gene; b) hypermutated microsatellite instability (MSI), characterized by mutations in the MMR genes: *MLH1*, *MSH2*, *MSH6*, *PMS2*; c) low copy number (LCN), which does not have a specific mutation; and d) high copy number (HCN), comprising almost all serous tumors and characterized by *TP53*<sup>146</sup> mutation. In this study, patients with *POLE* mutation had an excellent prognosis, whereas patients in the HCN subgroup had the worst prognosis. MSI and LCN patients had an intermediate prognosis. The TCGA findings were replicated by large studies in Vancouver (Canada) and Leiden (Netherlands) using more accessible methods, such as IHC for p53 and MMRd, and sequencing for *POLE*<sup>148-151</sup>.

Patients in the *POLE* and MSI groups are considered to have hot tumors with high neoantigen formation, high TMB and, therefore, are excellent candidates for immunotherapy<sup>146</sup>. Several studies have shown activity of anti-PD1 (e.g. pembrolizumab and dostalimab) and anti-PD-L1 (e.g. atezolizumab and avelumab) agents with global response rates ranging from 25 to 50% for patients with MSI<sup>151-154</sup>. In a recent analysis of PORTEC 3, patients with *TP53* mutation had benefits with combined treatment with chemoradiotherapy, particularly when compared to radiotherapy alone. In the PORTEC 2 study, patients with a *TP53* mutation had better survival when treated with pelvic radiotherapy compared to brachytherapy<sup>155</sup>.

The HCN subgroup presents *ERBB2* amplification in approximately 25% of patients<sup>146</sup>. In a randomized phase II study, patients with stage III/IV or relapsed serous carcinoma and HER2 expression in IHC (based on the ASCO/American College of Pathology 2007 guidelines) had better progression-free survival (PFS) and global survival (OS) when trastuzumab was added to carboplatin and paclitaxel<sup>156,157</sup>.

Patients with positive hormone receptor EC appear to be more likely to respond to endocrine therapy. In

**Table 3.** Main somatic alteration in cancer of gastrointestinal cancer.

Type of cancer	Gene / Alteration*	Test
All (agnostic approval)	Microsatellite instability (MSI-high) <i>NTRK 1-3</i>	Microsatellite instability assessment by IHC, RT-PCR or NGS IHC pan-TRK as a screening test for NTRK 1-3 fusion; if positive, confirm with NGS
Esophageal and gastric adenocarcinoma	Microsatellite instability (MSI-high)  <i>EBR2</i>	Microsatellite instability assessment by IHC, RT-PCR, or NGS  HER-2 amplification assessment by IHC and, if uncertain, confirm with FISH
Biliary tract adenocarcinoma	<i>FGFR2</i> (fusion) <i>IDH1</i> <i>BRAF</i> (V600E) <i>ERBB2</i> (amplification)	NGS IHC for HER-2 and, if uncertain, FISH
Colorectal adenocarcinoma	Microsatellite instability (MSI-high)  <i>KRAS e NRAS</i> <i>BRAF</i> (V600E) <i>ERBB2</i> <i>POLE</i>	In stage II, assessment of MSI by IHC, RT-PCR, or NGS In the metastatic disease, request NGS panel for RAS-RAF pathway  IHC for HER-2 amplification and, if uncertain, FISH
Neuroendocrine tumors	<i>BRAF</i> (V600E)	NGS in the metastatic disease, G3/Neuroendocrine carcinoma (NEC)

\* The frequencies can vary significantly depending on the tumor.

a randomized study, the response rate observed in patients with positive RE and PR was 25% and 37%, respectively, but the response rate was only 7% to 8% in patients with negative RE/PR disease<sup>158,159</sup>. Hormone therapy is the preferred systemic treatment for patients with grades 1 or 2 RH positive tumors and the absence of rapidly progressive disease<sup>160</sup>.

L1CAM is an adhesion protein that has been recognized as an adverse prognostic marker in EC. In a multicenter study with 1,021 patients with endometrial cancer, L1CAM positive tumors had worse PFS and OS<sup>161</sup>.

### Ovarian Cancer

What should be assessed in a somatic mutation panel (which genes should be included and what alterations are expected from each gene)?

1. In patients with stages III and IV or relapsed non-mucinous epithelial ovarian carcinoma (EOC) and *BRCA1/2* germline mutation negative, the assessment of somatic mutation should be performed by NGS (recommendation type: evidence-based; recommendation strength: strong);

2. Patients with endometrioid, mucinous, and clear cell carcinomas should perform MMRd screening by sequencing the *MLH1*, *MSH2*, *MSH6*, and *PMS2* genes by NGS, or by IHC for the respective proteins (type of recommendation: evidence-based; strength of recommendation: moderate);

How? Which platforms or tests are the most appropriate?

3. Sequencing should use a multigenic panel, including at least the *BRCA1*, *BRCA2*, *RAD51C*, *RAD51D*, *BRIP1*, *MLH1*, *MSH2*, *MSH6*, *PMS2*, and *PALB2* genes (recommendation type: evidence-based; recommendation strength: moderate);

4. NGS is the recommended method for somatic sequencing of *BRCA1/2* in paraffin. The choice for evaluating MMRd alone is IHC, an accessible and low-cost method (NGS panels may also be used) (type of recommendation: evidence-based; strength of recommendation: strong).

### DISCUSSION

Pathogenic variants in one of the BRCA genes are found in about 21% of patients with EOC: 14% are germline mutation and 7% are somatic mutation. Defects in DNA repair secondary to deficiency of homologous recombination (HR) pathways are detected in about 50% of patients. HR, particularly by *BRCA* mutation, is associated with response to PARP inhibitor (PARPi) therapy<sup>162</sup>.

Four randomized studies support the use of this drug class in the first line of patients with EOC. The SOLO-1 study demonstrated the benefit of olaparib maintenance therapy following first line in patients with *BRCA1/2* mutation and partial or complete response to platinum. It was observed reduced risk of progression or death by 70% (95% CI: 0.23 -0.41; p <0.0001)<sup>163</sup>. These results supported the approval of olaparib in this scenario in Brazil.

The PRIMA, VELIA, and PAOLA-1 studies evaluated, respectively, maintenance with niraparib<sup>164</sup>, veliparibe concomitant with chemotherapy followed by maintenance for three years<sup>165</sup>, and olaparib maintenance for two years in association with bevacizumab<sup>166</sup>. The three studies included patients independent of the *BRCA* status. The greatest benefit observed with the use of PARPi was observed in the population with a *BRCA* pathogenic variant (HR: PRIMA 0.4; VELIA 0.44; PAOLA 0.31). The presence of HR (VELIA cut-off  $\geq 33$ ; PRIMA and PAOLA-1 cut-off  $\geq 42$ ) without *BRCA1/2* mutation was also associated with greater response to PARPi in the PRIMA and PAOLA-1 studies, but not in the VELIA study; only the PRIMA study showed benefits for patients with HR proficiency. Veliparibe and niraparib are not yet approved in Brazil.

The SOLO-2 study and the 19 study support the use of olaparib in patients with relapsed and platinum-sensitive (PS) EOC. The first included patients with *BRCA1/2* mutation, after at least two platinum-based therapeutic lines and with partial or complete response, leading to a significant increase in OS<sup>167</sup>. The second study was a phase 2 study that included patients regardless of the presence of *BRCA1/2* mutation, and it was the first study to demonstrate increased OS with olaparib in relapsed EOC<sup>168</sup>. These studies supported the olaparib approval in Brazil in this scenario, regardless of the presence of *BRCA1/2* mutation.

Similar increases in PFS were obtained with PARPi niraparib and rucaparib, drugs not yet available in Brazil. The OS SG data from the ARIEL3 and NOVA studies are not yet concluded<sup>169</sup>. NCCN and ESMO recommend the use of PARPi in relapsed EOC PS, regardless of the *BRCA* mutation status<sup>170,171</sup>. The predictive value of HR is still debated, but it is gaining attention. In 2019, the myChoice platform was approved for the use of niraparib in recurrent EOC PS and, recently, for the use of olaparib combined with bevacizumab in the first-line treatment of patients without *BRCA* mutation. It is important to mention that this platform is not yet available in Brazil.

In an unselected manner, 10-12% of epithelial ovarian cancer may have dMMR<sup>172</sup> and, although pembrolizumab has not received agnostic approval in Brazil, these patients are potential candidates for immunotherapy.

### Breast Cancer (BC)

When should a somatic panel test be requested and for which patients? When is the best time to request?

1. The first-line treatment used in metastatic breast cancer (MBC) is supported by extensive literature. The use of panels may only be considered in patients with MBC who need additional cancer treatment - particularly when standard/registered treatment options are limited. It is crucial that patients and their families understand that somatic panels provide useful results in only a minority of patients. Often, access to the recommended treatment is very restricted - since most treatments based on these

panels are not approved by Brazilian regulatory agencies and, therefore, are not available, either in public health or supplementary health. There is also a very limited number of clinical studies that are based on the results of somatic panels in our country. Families should also understand that, in general, they may need to pay for the panels themselves and any treatment. Therefore, this document should not be used as a justification for requesting health plans or the public health system, whether by judicial or other means.

Another potential use of the panels is the assessment of multiple biomarkers required (for registered treatments) in a single test than the use of individual and sequential testing of a rapidly growing number of biomarkers.

What should be assessed in a somatic mutation panel (which genes should be included and what alterations are expected from each gene)? How? Which platforms or tests are the most appropriate?

2. It is suggested to assess somatic genomic alterations with a strong level of evidence for an intervention, including *ERBB2* amplification, activating *PIK3CA* mutation, microsatellite instability, *NTRK1*, *NTRK2*, and *NTRK3* fusion, and high mutational tumor burden - TMB). All of these alterations are predictive of benefits in different therapies and are approved by the FDA. In addition, it is suggested to assess *BRCA1* or *BRCA2* somatic mutation and *ERBB2* mutation when they have a weak/moderate level of evidence for modification of clinical management.

There are still few situations in which the identification of specific mutations based on somatic panels leads to the availability of other therapies with documented clinical benefit - in addition to case reports or small case series. In Brazil, where the availability of phase I and II clinical studies is limited, the performance of somatic panels in patients with MBC must be very careful, since it will only rarely expand treatment options outside a research environment.

The MBC treatment continues to be mostly decided based on the evaluation of hormone receptor expression (by IHC), HER2 overexpression (by IHC) and/or amplification (by ISH - in situ hybridization), identification of *PIK3CA* activating mutation (by PCR, in 3 hotspots), PD-L1 expression (by IHC), *BRCA1*, *BRCA2*, and possibly *PALB2* germline mutation (by genomic sequencing of the DNA host in blood or saliva), microsatellites instability (by IHC and/or PCR) and *NTRK* fusion (PCR or NGS). By the way, many of these tests are provided free of charge by drug manufacturers.

It is important to mention that, for the assessment of the aforementioned genomic alterations, it is possible to perform individual tests, with reliability similar to the use of somatic panels with wide coverage and at a lower cost. However, with the increasing number of targets to be assessed, issues regarding the depletion/circulation of biological material must be considered, since the panels represent a practical al-

ternative to testing multiple biomarkers individually. Even for this purpose, it is emphasized that there is still no health insurance coverage in the country.

## DISCUSSION

Advanced cancer has gone through changes during the disease and, thus, the genomic profile becomes more complex than that of early BC<sup>173</sup>. Although sequencing is traditionally performed on tumor tissue, which is limited by the availability of the sample and the biopsy risk, the use of tumor DNA sequencing in plasma is an alternative with increasing use<sup>174</sup>.

It is important to assess whether the alteration found corresponds to targeted therapy and whether it results in a clinically relevant antitumor effect.

The identification of genomic alterations related to sensitivity and resistance can help in the selection of treatments for MBC. Although advanced sequencing methods have enabled to detect important genomic alterations, before considering the test, it is essential to determine whether sequencing is clinically recommended and how the results would affect treatment decisions. In addition, the evidence associated with treatment decisions based on genomic alterations discovered in the sequencing needs to be

continuously and critically evaluated<sup>174</sup>. Efforts have been made to create a comprehensive classification scheme that guides and prioritizes goals according to the level of evidence<sup>175</sup>.

*ERBB2* amplification (HER2): determines the HER2+ subtype and is widely validated as a predictor of response to anti-HER2 therapies: trastuzumab, pertuzumab, T-DM1, lapatinib, neratinib, trastuzumab deruxtecan, and tucatinib. Prospective randomized studies demonstrated an increase in overall survival (OS) and progression-free survival (PFS) in patients with MBC and amplification<sup>176</sup>. Although the use of next-generation sequencing and the use of somatic panels can detect *ERBB2* amplification, it is more commonly detected in clinical practice using IHC or fluorescent or chromogenic in situ hybridization (when IHC is uncertain).

*PIK3CA* mutation: about 40% of hormone-positive MBC have activating *PIK3CA* mutation, which codes the alpha chain of the PI3K protein. The randomized phase III SOLAR-1 study demonstrated the clinical relevance of *PIK3CA* mutation in hormone-positive MBC. In that study, patients with the *PIK3CA* mutation treated with alpelisib (an alpha-selective PI3K inhibitor) and fulvestrant had a median PFS of 11 months

**Table 4.** Main somatic alterations in endometrial and ovarian cancer.

Type of cancer	Gene / Alteration	-	Comments
All (agnostic approval)	<i>Microsatellite instability (MSI-high)</i>	Microsatellite instability assessment by IHC, RT-PCR, or NGS	
	<i>NTRK 1-3</i>	IHC pan-TRK as screening test for NTRK 1-3 fusion; if positive, confirm with NGS	
Ovarian Cancer (Stages III and IV)	<i>BRCA1, BRCA2, RAD51C, RAD51D, BRIP1, and PALB2 (BRCA1 ~ 8% germline and 3% somatic; BRCA2 ~ 6% germline and 3% somatic)</i>	NGS with a panel that includes genes associated with DNA repair by homologous recombination	The assessment of mutation in repair genes by homologous recombination should be carried out in germinating DNA and, if not, by sequencing the somatic DNA
	<i>MLH1, MLH2, MSH6, PMS2 (MSI ~ 12%, not selected by histology; endometrioid ~ 20%, mucinous ~ 17%, and clear cells ~ 12%)</i>	Microsatellite instability assessment by IHC, RT-PCR, or NGS in endometrioid, mucinous and clear cell carcinomas	
Endometrial cancer	<i>POLE (~7%)</i>	NGS	
	<i>MLH1, MLH2, MSH6, PMS2, p53 (MSI~30%; p53~25%)</i>	Microsatellite instability and p53 assessment by IHC, RT-PCR, or NGS	
	<i>L1CAM (~17%)</i>	IHC	
	<i>HER2 (overexpression in ~25% of serous tumors)</i>	IHC	
	<i>Hormonal receptors (estrogen and progesterone)</i>	IHC	

versus 5.7 months in the arm that received placebo and fulvestrant (HR 0.65;  $p = 0.00065$ ). There was no difference between groups in the cohort without *PIK3CA* mutation<sup>177</sup>.

**Microsatellite instability:** the incidence in BC is estimated to be around 1%<sup>178</sup>. Tumors with a deficiency in the repair system by unpaired bases recombination are more responsive to PD-1 blockade by pembrolizumab<sup>103</sup>. This drug has received agnostic approval in the USA (regardless of histology), based on the analysis of 149 patients (2 with BC) included in five prospective, single-arm cohort studies. The objective response rate was 39.6%. The two patients with BC had a partial response. Although there are methods of detecting this alteration using NGS, it is important to recognize that the current gold standard for detection is PCR or IHC<sup>179</sup>.

***NTRK* fusion:** Tropomyosin receptor kinase (TRK) family is composed of three transmembrane proteins (TrkA, TrkB, and TrkC), which are coded by the *NTRK1*, *NTRK2* and *NTRK3* genes, respectively. Chromosomal alterations that lead to fusions of different genes with *NTRK* genes determine the transcription of chimeric TRK proteins with kinase function, activated or overexpressed, giving oncogenic potential to these cells<sup>180</sup>. Currently, two TRK inhibitors are approved: Larotrectinib, and entrectinib (not approved in Brazil). Larotrectinib demonstrated effectiveness in the LOXO-101 study. In this study, 55 patients were included and treated with larotrectinib, including one patient with BC (2%). The objective response rate was 75%. After one year, 71% of the patients who had objective response remained with the response and 55% of the patients remained without progression. The median duration of the response and the median PFS have not been reached<sup>32</sup>, however, the frequency of *NTRK* fusion in the BC is very low; a study that evaluated 12,214 consecutive patients with MBC found that 0.13% of the tumors harbored *NTRK* fusion<sup>181</sup>. Among the BC subtypes, it is important to note that *NTRK* fusions are most commonly found in mammary analog secretory carcinoma (carcinoma of the salivary glands) and in secretory breast carcinoma<sup>182</sup>. It is important to note that the *NTRK* fusion has multiple partners and not all are oncogenic. In addition, *NTRK1* G595R, and *NTRK3* G623R hotspot mutations are probably associated with resistance to larotrectinib<sup>32</sup>.

**High tumor mutational burden:** the FDA recently approved the use of pembrolizumab for solid tumors with a high tumor mutational burden<sup>183</sup>. This approval is also considered agnostic and is based on a mutational burden > 10 mutations per megabase (mut / Mb), determined by the FoundationOne CDx somatic panel (Foundation Medicine, Inc.). The approval of this treatment was based on a retrospective analysis of the KEYNOTE-158 study, which included ten cohorts of tumors treated with pembrolizumab 200 mg every three weeks. Tumors with high mutational burden were present in 102 patients (13%). The objective response rate was 29%, the complete response

rate was 4%, and the partial response rate was 25%. The median duration of response was not achieved, as 57% of the patients had a duration of response  $\geq 12$  months and 50% of the patients had a duration of response  $\geq 24$  months. It is important to note that no patient with MBC was included in this analysis. In the MBC scenario, different groups have pointed out a prevalence of a high tumor mutational burden around 10%<sup>184-186</sup>. Some preliminary studies suggest a benefit in PFS and OS with the use of checkpoint inhibitors in these patients<sup>187,188</sup>.

Below are mentioned alterations with a weaker level of evidence, but with potential utility, depending on more scientific data:

***BRCA1/2* somatic mutations:** as long as there is robust evidence about the fact that *BRCA1* or *BRCA2* germline mutations predict benefits in using PARP inhibitors<sup>189,190</sup> or platinum agents<sup>191</sup>, the data about somatic mutations are still preliminary. Recently, Tung and colleagues presented the results of the TBCRC 048 study, a phase II, single-arm study that showed a response rate of 50% with the use of olaparib as monotherapy for patients with somatic mutations in one of these two genes<sup>192</sup>. It should be noted that somatic panels may not capture all *BRCA1/2* and *PALB2* germline mutations (possibly in 10-20% of patients)<sup>193</sup>, and these patients may respond very well to PARP inhibitors<sup>192</sup>.

***ERBB2* mutation:** *ERBB2* alteration, in addition to amplification, represent up to 20% of the total *ERBB2* alterations in these panels (and 2-3% of BC patients), and it is not detected by conventional IHC or FISH<sup>194</sup>. Preliminary data suggest possible response to anti-HER2 therapies<sup>195</sup>.

Despite these potential and uncommon benefits, prospective clinical studies that have attempted to assess the impact of these methods have failed<sup>196-198</sup>. The main justifications are the intratumoral heterogeneity, the lack of effective drugs for most of the molecular targets until now, the heterogeneous patient populations, and the previous and intense treatment of the vast majority of recruited patients. In addition, the studies may have selected patients with tumors that developed several resistance mechanisms.

Considering these data and the aforementioned exceptions, it is concluded that the use of somatic panels in MBC remains largely restricted to clinical research. The ASCO positioning and the ESMO Advanced Breast Cancer (ABC) guidelines<sup>4</sup> affirm that multigene panels should not be used in clinical practice for MBC<sup>199</sup>. However, the somatic panel may be used in prospective molecular screening programs that include patient selection for clinical trials, or as a practical substitute for testing multiple individual markers<sup>199</sup>.

## Genitourinary Cancer

### Prostate Cancer

When should a somatic panel test be requested and for which patients? When is the best time to request?



### Localized disease

1. Somatic testing may be offered to patients diagnosed with low-risk or favorable intermediate-risk localized prostate cancer. The ideal moment to offer the test is at the time of histopathological diagnosis, before initiating the treatment (type of evidence: evidence-based; strength of recommendation: weak);

2. Although many genes alone correlate with the prognosis in patients with localized disease, there is no validation for change in clinical practice based on specific gene alterations (type of evidence: evidence-based; strength of recommendation: moderate).

### Advanced disease

3. Somatic alterations testing should be offered to patients with metastatic castration-resistant prostate cancer (mCRPC). The ideal moment to offer the test is at the time of diagnosis of metastatic disease castration-resistant (type of evidence: evidence-based; strength of recommendation: strong).

What should be assessed in a somatic mutation panel (which genes should be included and what alterations are expected from each gene)? How? Which platforms or tests are the most appropriate?

4. Sequencing by NGS: Deleterious changes in genes responsible for DNA repair have prognostic value and can predict responses to different therapies. Changes in genes of the homologous recombination (HR), including *BRCA1*, *BRCA2*, *ATM*, *BRIP1*, *BARD1*, *CDK12*, *CHEK1*, *CHEK2*, *FANCL*, *PALB2*, *PPP2R2A*, *RAD51B*, *RAD51C*, *RAD51D*, and *RAD54L*, and deleterious alterations in the genes involved in the mismatch repair pathway (dMMR), including *MSH2*, *MSH6*, *PMS2*, and *MLH1*, must be included on NGS panels for patients with mCRPC (type of evidence: evidence-based; strength of recommendation: strong);

5. Gene alterations, such as *PI3K*, *AKT*, *PTEN*, *TP53*, *RB1*, *CTNNB1*, *APC*, and *RNF43* also have prognostic value and, therefore, should be included, preferably, in NGS panel. These tests can be performed from paraffin-embedded tissue adequately conserved, however, preference should be given, if feasible, to recent metastatic lesion biopsy or circulating tumor DNA, since these reflect more reliably the tumor molecular growth status. Other non-molecular biomarkers may also have a prognostic value, among them the most relevant is the presence of variant 7 of the androgenic receptor (AR-V7), a biomarker with predictive value for resistance to antiandrogenic therapy and also related to the worse prognosis (type of evidence: evidence-based; recommendation strength: weak);

6. For assessment of DNA repair defects in the homologous recombination (HR) pathway, validated multigenic panels are always more complete than the evaluation of single genes (*BRCA1* or *BRCA2*, for example), since the targeted therapy was approved based on a panel with 15 genes from the HR pathway in this scenario. Therefore, the assessment of single

may deprive many patients of effective therapy. In patients with suspected microsatellite instability tumor, IHC assessment of loss *MSH2*, *MSH6*, *PMS2*, and *MLH1* expression can adequately replace NGS panel (type of evidence: evidence-based; strength of recommendation: strong).

### DISCUSSION

Patients with localized prostate cancer can benefit from molecular tests, both for prognostic stratification and treatment selection<sup>200,201</sup>.

Although therapeutic decisions in patients with localized prostate cancer are based on clinical (PSA, clinical stage) and pathological (Gleason score) factors, some molecular tests may help in the management of patients with low-risk disease and in some patients with favorable intermediate-risk<sup>200,201</sup>, that are candidates for active surveillance. Molecular diagnostic tests such as Decipher, 202 OncotypeDx Prostate203, and Prolaris204 are commercially available and, in selected cases, may help in the management of localized prostate cancer<sup>200,201</sup>. Not all diagnostic tests are available in Brazil and, due to the lack of comparisons between them, the most easily and available test should be prioritized. Despite the multiple options for testing in patients with localized disease, none of them has established itself as the gold standard and, thus, therapeutic decisions based on clinical and pathological factors are still the standard practice.

Some gene alterations are associated with worse outcomes in patients with localized prostate cancer, such as *BRCA2* mutation and alterations in genes responsible for the repair of mismatch (*MSH2*, *MSH6*, *PMS2*, and *MLH1*) that are present in about 3-8 % of these patients. DNA repair gene alterations are associated with disease progression, shorter metastasis-free survival, shorter time to start hormone therapy, and worse overall survival<sup>205-207</sup>. Patients with the higher Gleason score<sup>208</sup>, primary Gleason 5 (5 + 4 and 5 + 5)<sup>209</sup>, ductal histology<sup>96</sup>, more advanced stage<sup>206</sup>, lymph node involvement<sup>206</sup>, angiolymphatic invasion<sup>96</sup>, and metastases at diagnosis<sup>206</sup> are more likely to have these deleterious genetic alterations. Although there is still no recommendation for change in clinical practice in the presence of these alterations, clinical studies with targeted therapies may be offered to patients with these genetic abnormalities<sup>200,201</sup>.

In advanced disease, some molecular changes have gained attention in the last years, particularly in genes responsible for DNA repair - which are more common in patients with castration-resistant prostate cancer (CRPC). Approximately 23%<sup>210</sup> of patients with CRPC have somatic alteration in these genes and 12% have germline alteration<sup>211</sup>. The two DNA repair pathways with alterations seen in patients with CRPC are the homologous recombination (HR) pathway and the mismatch pathway (dMMR). In advanced disease, changes in these pathways have a relevant therapeutic role. Patients with HR pathway alteration are candidates for PARP inhibitors thera-

**Table 5.** Main somatic alterations in breast cancer.

Gene / Alteration	Test	Comments
<i>PIK3CA</i> (~40%)	RT-PCR or NSG for <i>PIK3CA</i> , in cfDNA or tissue sample	It is a standard test for patients with RH + HER2- tumors
Germline <i>BRCA1</i> or <i>BRCA2</i> (up to 10%)*	NGS may be useful for identifying potential germline mutation of these genes	This is a standard test for patients with metastatic breast cancer This test must be restricted and individualized due to the lack of pivotal studies that define management
Germline <i>PALB2</i> mutation (~2%)*	NGS may be useful for identifying potential germline mutation of these genes	This test must be restricted and individualized due to the lack of pivotal studies that define management
<i>TMB</i> (~10% in triple negative; <5% in luminal)	NGS may be useful for determining the tumor mutational burden	
Somatic <i>BRCA1</i> or <i>BRCA2</i> (~5-10%)	NGS may be useful for determining the somatic mutation in these genes	
<i>PD-L1</i> (~2-6% for amplification, ~20-25% including copy number gain)	NGS may be useful for determining the copy number gain/amplification of this gene	In metastatic disease, the somatic NGS test is restricted and individualized due to the lack of pivotal studies that define management
<i>MSI</i> (~1%)	NGS or RT-PCR may be useful for determining microsatellite instability	
<i>NTRK1/2/3</i> (<0,5%)	NGS or RT-PCR may be useful for determining <i>NTRK</i> fusion	
<i>ERBB2</i> (~2-3% for mutation and ~10% for amplification)	NGS may be useful for determining <i>ERBB2</i> amplification or mutations - with additional information regarding HER2 status already determined by standard IHC / FISH	

\* When identified in the somatic panel, gene mutation must be confirmed in tests in blood or saliva to evaluate germline mutations. Particularly related to *BRCA1* and *BRCA2* genes, even somatic mutations are associated with the response to treatment with PARP inhibitors.

py<sup>212-214</sup> and patients with mismatch repair deficiency (dMMR) are candidates for PD1 inhibitors therapy<sup>151</sup>.

The phase III PROFound study evaluated olaparib in patients with CRPC<sup>213</sup>. Patients with deleterious alterations in genes related to the HR pathway and who had disease progression receiving antiandrogenic therapy with abiraterone or enzalutamide were included. Olaparib demonstrated benefit in progression-free survival by imaging (primary outcome), both in cohort A (*BRCA1*, *BRCA2*, and *ATM* alteration) and in the general study population (including other alterations related to the HR pathway). Cohort A patients who received olaparib showed benefit in overall survival, demonstrating that this therapy can increase survival in selected patients<sup>215</sup>. Despite being analyzed as one, each type of genetic alteration in the HR pathway probably is associated with a different benefit from PARP inhibitor olaparib therapy. Each patient must be individualized, weighing the risks and benefits.

Several retrospective series suggest that defects in the DNA repair by HR pathway have also been associated with better responses with the use of radium-223<sup>216,217</sup> and platinum-based chemotherapy<sup>218</sup>.

However, these findings must be interpreted with caution until they are validated in prospective studies.

The benefit of using pembrolizumab in patients with CRPC is derived from this drug as agnostic therapy in patients with defects in the mismatch repair pathway<sup>151,219</sup>, which have alteration in up to 8% of patients with CRPC<sup>207</sup>. Despite preliminary data showing a benefit with the use of PD1 inhibitors in patients with *CDK12* mutation<sup>220</sup>, new studies with a greater number of patients did not confirm that the mutation in this gene is a biomarker of response to immunotherapy<sup>218,221</sup>.

Some genes that are included in most commercially available NGS panels may provide prognostic information and information related to resistance to some therapies. Genes such as *PI3K*, *AKT*, *PTEN*, *TP53*, *RB1*, *CTNNB1*, *APC*, and *RNF43* are associated with worse prognosis and resistance to antiandrogenic therapies. Despite being clinically relevant, these data should be interpreted with caution until validated in prospective studies<sup>222-225</sup>.

From the data exposed above, patients with prostate cancer, at different times of the disease, may benefit from somatic molecular tests for both prognostic information and treatment selection<sup>201</sup>.

## Urothelial Carcinoma

When should a somatic panel test be requested and for which patients? When is the best time to request?

1. Patients with advanced urothelial carcinoma (stage IV), preferably during the first line of treatment or shortly after its failure. Tests can be carried out using paraffin-embedded tissue in good condition (type of recommendation: formal consensus; strength of recommendation: strong).

What should be assessed in a somatic mutation panel (which genes should be included and what alterations are expected from each gene)?

2. Target with regulatory approval: *FGFR2* and *FGFR3* alterations (mutations and fusions) (type of recommendation: evidence-based; strength of the recommendation: strong);

3. Biomarkers (non-molecular) with regulatory implications: PD-L1 expression (antibodies 22C3 or SP142 by IHC) (type of recommendation: evidence-based; strength of recommendation: moderate);

4. Other potential therapeutic targets for inclusion in clinical studies: HER-2, *TSC1*, DNA repair genes (type of recommendation: evidence-based; strength of recommendation: weak).

How? Which platforms or tests are the most appropriate?

5. RT-PCR (Real-time PCR) for *FGFR* Therascreen (QIAGEN) (preferred test). Commercially available or through sponsored testing programs (recommendation type: evidence-based; recommendation strength: strong);

6. NGS panel: multigenic panels available on the market (check whether the *FGFR2* and 3 genes are included in the panel, including fusions and mutations - give preference to panels that include these alterations) (type of recommendation: formal consensus; strength of recommendation: weak).

## DISCUSSION

Patients with advanced urothelial carcinoma may benefit from somatic molecular tests for treatment selection. Ideally, requesting these tests should be considered in patients with stage IV disease, preferably before or during the first line of treatment. Currently, there is no evidence to support the selection of therapies based on molecular tests in non-metastatic tumors. However, it is important to consider that some urothelial carcinomas show rapid progression, and there is no time to perform tests in advanced stages of the disease. Clinical studies are available with targeted therapy at earlier stages, which may justify performing specific tests in this scenario.

Currently, the main therapeutic targets available with regulatory approval for treatment are *FGFR2* and *FGFR3* alterations (mutations and fusions). Patients with these alterations may be treated with the erdafitinib, which was approved by ANVISA for patients

who had a failure in at least one previous treatment line in metastatic disease. This approval is based on phase II clinical study that demonstrated an objective response rate of 40%, progression-free survival of 5.5 months, and overall survival of 13.8 months with erdafitinib<sup>226</sup>. In addition, there are currently open clinical studies in Brazilian centers for patients with *FGFR* alterations. It is important to remember that the *FGFR* amplification or FGF ligand can be detected in some NGS platforms, but it does not have predictive value for the use of *FGFR* inhibitors.

The PD-L1 expression, although not part of the molecular analysis, is an important biomarker for determining the first line of treatment in urothelial carcinoma. Patients who are not candidates for cisplatin and whose tumors express PD-L1 are candidates for the use of PD-1/PD-L1 inhibitors in the first line of treatment<sup>227,228</sup>. It is important to mention that this biomarker is not necessary when choosing to use these drugs in later lines of treatment. In the use of immune checkpoint inhibitors in urothelial tumors, molecular markers, such as the tumor mutational burden (TMB) or the presence of microsatellite instability (MSI), are not necessary, which may be assessed on NGS platforms. However, several studies demonstrate that high TMB or MSI-high correlates with a greater probability of response to immunotherapy<sup>229</sup>, which may be useful information for the therapeutic decision, depending on the clinical scenario.

There are other molecular changes in urothelial carcinoma that can be classified as Tier 2, that is, investigational targets with some clinical evidence of benefit<sup>230</sup>. These targets can be assessed in patients with good clinical conditions for inclusion in studies. Among them, we mention the *TSC1* mutation (prevalence of approximately 8%), which may correlate with responses to mTOR<sup>231</sup> inhibitors; in *ERBB2* and *ERBB3* mutation and amplification (prevalence of approximately 15%), which can predict response to anti-HER2 drugs<sup>232</sup>; and changes in DNA repair genes (DDR), which may indicate activity of PARP inhibitors<sup>233</sup>.

Regarding the consensus recommendations, according to the European Society of Clinical Oncology (ESMO), there is no consensus on the performance of molecular tests in advanced urothelial carcinoma, including the markers that should be assessed; however, there is a consensus against not considering this type of assessment depending on the scenario<sup>234</sup>. The NCCN recommends performing molecular tests for IVA and IVB stages, particularly *FGFR* analysis by RT-PCR ([https://www.nccn.org/professionals/physician\\_gls/pdf/bladder.pdf](https://www.nccn.org/professionals/physician_gls/pdf/bladder.pdf)).

Thus, currently, the molecular test to be considered in advanced urothelial carcinoma is the evaluation of *FGFR2* and -3, which defines the recommendation for the use of *FGFR* inhibitor. Other molecular panels can be considered for the inclusion of patients in clinical studies.

## Kidney Cancer

When should a somatic panel test be requested and for which patients? When is the best time to request?

1. There is no recommendation to request somatic molecular tests in patients with renal cell carcinoma. Such recommendation applies to localized or metastatic disease (type of recommendation: informal consensus; strength of recommendation: strong). Molecular tests can be performed to include patients in clinical studies.

What should be assessed in a somatic mutation panel (which genes should be included and what alterations are expected from each gene)? How? Which platforms or tests are the most appropriate?

2. There is no targeted therapy with regulatory approval in renal cell carcinoma (type of recommendation: evidence-based; strength of recommendation: strong);

3. Tests and platforms available: not applicable.

### Solid Hematologic Malignancies

Only the most frequent lymphomas are addressed here with the name solid hematological tumors. There are dozens of lymphoma subtypes. The two main categories of lymphomas are B-cell lymphomas and T-cell lymphomas (not covered in this text). In addition, lymphomas can also be divided between Hodgkin's Lymphomas (HL) and Non-Hodgkin's Lymphomas (NHL). About 90% of lymphomas are NHL and, among them, diffuse large B-cell lymphoma and follicular lymphoma are the most frequent.

### Diffuse large B-cell lymphoma (DLBCL)

When should a somatic panel test be requested and for which patients? When is the best time to request?

1. All DLBCL patients should be tested for COO (cell-of-origin) classification (type of recommendation: evidence-based; strength of evidence: strong);

2. The assessment for somatic mutation panel with NGS and the identification of DLBCL subgroups based on these changes, although studied in a large number of cases, are not yet validated in clinical practice and therefore should not be used in the clinical practice for decision-making (type of recommendation: formal consensus; strength of evidence: moderate).

What should be assessed in a somatic mutation panel (which genes should be included and what alterations are expected from each gene)? How? Which platforms or tests are the most appropriate?

1. Hans's algorithm, testing CD10, BCL6, and MUM1 by IHC, may be used in COO (type of recommendation: formal consensus; strength of evidence: moderate);

2. The routine use of gene expression profile (GEP) for the definition of COO is not recommended. The use of IHC algorithms is allowed, although the definition of

COO is more accurate by GEP (type of recommendation: evidence-based; strength of evidence: strong);

3. Whenever possible, request FISH for *MYC*, *BCL2*, and *BCL6* translocation assessment (type of recommendation: formal consensus; strength of evidence: strong);

4. The *MYC* and *BCL2* double expression by IHC should be assessed in all patients. In the absence of translocation (negative FISH), these patients should be reported as "double expressors" (type of recommendation: evidence-based; strength of evidence: strong);

5. It is reasonable to assess *MYC* isolated translocation initially and, if positive, proceed with *BCL2* and *BCL6* (type of recommendation: informal consensus; strength of evidence: moderate);

6. In situations of difficulty in carrying out FISH, prioritize patients with higher immunoexpression rates: 40% for *MYC* and 50% for *BCL2* (type of recommendation: formal consensus; strength of evidence: weak).

## DISCUSSION

The determination of the cell-of-origin (COO) is considered mandatory by the most recent version of the World Health Organization (WHO). COO can be determined using IHC. Hans's algorithm uses CD10, BCL6, and MUM1 expression, and can distinguish the types of germinal center (GC) and activated/unclassifiable B cell (not CG or ABC)<sup>235</sup>. When compared to gene expression profiling (GEP), Hans' algorithm has an accuracy of about 85-90%<sup>236</sup>. Due to its easy application, low cost, and good correlation, it is indicated for use in clinical practice. The distinction between CG and CBA is important since the last subgroup is associated with the worse prognosis<sup>237</sup>.

More recently, the role of *MYC* and *BCL2* protein expression by IHC has been associated with poor prognosis<sup>238</sup>, regardless of COO<sup>239</sup>. A positive result should be considered when it is greater than 40% for *MYC* and greater than 50% for *BCL2*. The scenario in which both are positive, but without genetic translocation, is called "double expressor". Based on the results of COO and *MYC* and *BCL2* expression by IHC, new drugs are being tested combined with standard chemotherapy protocol, R-CHOP, to try to improve the outcomes in this population<sup>240</sup>.

Studies using NGS have demonstrated the difference between the CG and ABC subtypes, in addition to discovering new mutations with prognostic and therapeutic potential. The most frequent alterations found in patients with CG subtype include the *BCL2* gene (34%, translocation and mutation), while those with ABC subtype include *TNFAIP3* (30%, mutation and deletion), and *MYD88* (30%, mutation)<sup>241</sup>. *BCL6* translocation (35%) and *KMT2D* mutation (35%) have similar frequencies in the two COO subtypes. After evaluating almost 600 biopsies of DLBCL<sup>242</sup>, four genetic subgroups were proposed: MCD (*MYD88* and *CD79B* mutation), BN2 (*BCL6* fusion and *NOTCH2* mutation), N1 (*NOTCH1* mutation), and EZB (*EZH2* mutation and *BCL2* translocation). The BN2 and EZB

**Table 6.** Main somatic alterations in urological tumors

Type of cancer	Gene / Alteration	Test	Comments
Prostate cancer (advanced)*	<i>Microsatellite instability (MSI-high) (3-4%)</i>	Microsatellite instability assessment by IHC, RT-PCR or NGS	Genomic panels can help in the decision to treat low-risk localized disease and in some patients with favorable intermediate-risk.
	<i>Genes associated with DNA repair by homologous recombination: BRCA1, BRCA2, ATM, BRIP1, BARD1, CDK12, CHEK1, CHEK2, FANCL, PALB2, PPP2R2A, RAD51B, RAD51C, RAD51D, RAD54L (23%)</i>	NGS (in the metastatic scenario, in castration-resistant disease)	
	<i>PI3K, AKT, PTEN, TP53, RB1, CTNNB1, APC, RNF43</i>		
Urothelial carcinoma	<i>AR (variant 7) (20%)</i>	RT-PCR for detection of variant 7 of the androgen receptor (AR) transcript	Assessment of PD-L1 by IHC is necessary for the use of first-line immune checkpoint inhibitors for patients not eligible for cisplatin
	<i>FGFR2 and FGFR3 mutation or fusion (20%)</i>	RT-PCR (Real-time PCR) for <i>FGFR</i> Therascreen (QIAGEN) NGS for targets beyond <i>FGFR</i>	

\* Frequency data are related to metastatic disease castration-resistant.

groups seem to have a better clinical outcome than the MCD and N1 groups.

Alterations in *MYC*, *BCL2*, and *BCL6* genes may result from different mechanisms<sup>243</sup>. Molecular abnormalities of these genes tend to produce more aggressive phenotypes of the disease, in the case of translocations, than by point or indel mutations. The most traditional method for the assessment these translocations is using in situ hybridization (FISH). Lymphomas that simultaneously host the aforementioned translocations are called Double Hit (*MYC* + *BCL-2*) or Triple Hit (*MYC* + *BCL-2* + *BCL-6*). High-grade B lymphomas with *MYC* and *BCL2* or *BCL6* translocation are recognized as a new entity by the recent WHO classification<sup>236</sup>. This group with a worse prognosis has a very poor response to conventional chemotherapy and there is still no consensus on how these patients should be approached<sup>244,245</sup>.

In situations of a scarcity of resources for genetic translocation assessment, it is possible to perform FISH initially only for *MYC*, reserving *BCL2* and *BCL6* assessment for situations in which the first one is positive<sup>246</sup>. Another marker with a possible prognostic role is IRF4/MUM1 by IHC. It has already been demonstrated that its expression may be associated with a higher response rate in subtype CBA<sup>247</sup>.

## FOLLICULAR LYMPHOMA (FL)

When should a somatic panel test be requested and for which patients? When is the best time to request?

1. Currently, a specific genetic panel is not recommended for patients with FL that helps in the assessment of evolution, therapeutic response, or risk of transformation to aggressive lymphoma (type of recommendation: formal consensus; strength of evidence: strong).

What should be assessed in a somatic mutation panel (which genes should be included and what alterations are expected from each gene)? How? Which platforms or tests are the most appropriate?

1. All patients with FL and uncertain diagnosis should be referred for direct assessment of t(14;18) (q32.3,q21.3), using a specific method (FISH) (type of recommendation: evidence-based; strength of evidence: strong);

2. The routine use of FLIPI-m7 is not recommended as a prognostic tool, since it is not validated in different cohorts of patients with FL (type of recommendation: evidence-based; strength of evidence: moderate);

3. The loss of *BCL2* translocation and activation-induced deaminase (AID) overexpression may be a

clue for the diagnosis of follicular lymphoma transformed to DLBCL (type of recommendation: informal consensus; strength of evidence: weak).

## DISCUSSION

The most characteristic genetic alteration of FL is the *BCL2* proto-oncogene translocation with the immunoglobulin heavy chain (IgH) gene locus<sup>248</sup>. The result is a reciprocal translocation t(14;18)(q32.3,q21.3), which results in *BCL2* constitutive overexpression and gives an anti-apoptotic effect to the tumor cell<sup>248</sup>. This translocation alone is not able to promote lymphomagenesis, requiring other changes to be added for this process to occur. Healthy individuals can have t(14;18)(q32.3,q21.3) in circulating B lymphocytes without developing the disease<sup>249</sup>. Among the molecular changes, the most important and frequent is the *KMT2D* (or *MLL2*) mutation, which occurs in 70-80% of cases<sup>250</sup>. In general, epigenetic changes are often seen in FL.

To gather data related to molecular changes with clinical data in a combined prognostic index, FLIPI-m7 was developed<sup>251</sup>. This prognostic score integrated the risk factors of the FLIPI (score that uses age, number of nodal sites, LDH value, hemoglobin, and Ann Arbor staging) to the performance status and added seven genes frequently mutated in the FL (*EZH2*, *ARID1A*, *MEF2B*, *EP300*, *FOXO1*, *CREBBP*, and *CARD11*). FLIPI-m7 was validated in patients treated with R-CHOP or R-CVP, classic first-line regimens in the treatment of the disease, but not in patients exposed to bendamustine or rituximab as monotherapy<sup>252</sup>. Patients with FL and disease progression within the first two years after first-line treatment with R-CHOP (POD24) have a worse prognosis, particularly when compared to patients with progression after two years<sup>253</sup>. In a study that prospectively evaluated FLIPI-m7 in patients with POD24, almost half of the population was classified as low risk, showing that this is not a sensitive tool to identify a group with worse outcomes.

The transformation of the FL into an aggressive lymphoma (histological grade 3B) is an event that occurs in about 10-15% of the cases<sup>254</sup> and represents one of the main causes of mortality related to the disease<sup>255</sup>.

When histological transformation occurs, the morphology resembles a new DLBCL in most cases. This phenomenon seems to be related to activation-induced deaminase (AID) overexpression<sup>256</sup> and loss of *BCL2* translocation<sup>236,257</sup>. However, so far there is no combination of mutations that can be used for the diagnosis of this process.

### Hodgkin's lymphoma (HL)

When should a somatic panel test be requested and for which patients? When is the best time to request?

1. Currently, no specific genetic panel is recommended for patients with HL to help in the evaluation of the therapeutic response or outcomes (type of recommendation: formal consensus; strength of evidence: strong).

What should be assessed in a somatic mutation panel (which genes should be included and what alterations are expected from each gene)? How? Which platforms or tests are the most appropriate?

1. Due to the high prevalence of the EBV virus in patients with HL, its assessment by protein expression (LMP1, IHQ) or direct expression of its genomic material (EBER, in situ hybridization) is recommended for assisting in the diagnosis of this lymphoma (type of recommendation: evidence-based, strength of evidence: strong);

2. The use of IHC to evaluate tumor PD-L1 expression and MHC class II positivity are predictors of favorable outcomes for patients treated with PD1 inhibitors (type of recommendation: formal consensus; strength of evidence: moderate);

3. Genetic evaluation for alterations associated with worse prognosis in patients treated with chemotherapy, such as chromosome 9p24.1 amplification, should not be routinely performed (type of recommendation: formal consensus; strength of evidence: moderate);

4. Assessment of hotspots with circulating tumor DNA (ctDNA), such as *STAT6* mutation, is still experimental in HL and should not be currently used in the management of cases in clinical practice (type of recommendation: formal consensus; strength of evidence: moderate)

## DISCUSSION

There is a close relationship between HL and EBV virus, that is present in tissue samples of lymph node biopsy<sup>258</sup>, as well as an inversely proportional association with common childhood infections, particularly measles, rubella, and mumps<sup>259</sup>. HL can be divided into classic HL and nodular lymphocytic-predominance<sup>236</sup>. The first is the most common type, representing about 90% of cases. The characteristic neoplastic cell is the Reed-Stenberg (RS) cell, which is derived from B cell and it is giant, multinucleated and with an inflammatory infiltrate around it<sup>260</sup>. Considering all tumor tissue, the RS cell represents between 0.1-10% and the other cells are lymphocytes, histiocytes, and peripheral reactional eosinophils<sup>236</sup>. The RS cell has increased expression of ligands 1 and 2 of PD-1 (programmed death-1), PD-L1, and PD-L2, which protects against the mechanisms of death induced by T lymphocytes (immune evasion phenomenon)<sup>261,262</sup>. Variation in the number of chromosomal copies is frequent in HL, particularly of the chromosome 9p24.1 (location of *JAK2*, *PD-L1*, and *PD-L2* genes), a frequent finding in patients with advanced disease and associated with reduced progression-free survival with chemotherapy<sup>261-263</sup>.

The most common genetic alterations in HL lead to changes in the three main signaling pathways: NF-κB (*TNFAIP3* mutation in about 40% of patients and more frequently in EBV+ patients), JAK / STAT (*SOC31* and *STAT6* mutation in 30-40% of patients) and MHC1 (*B2M* mutation in up to 70% of patients, particularly in the nodular lymphocytic predominant subtype)<sup>264</sup>. As the

number of neoplastic cells in comparison to the tumor tissue is very small, studies of genetic alterations in this lymphoma have always been quite challenging. The use of circulating tumor DNA (ctDNA) is increasing in HL and Italian authors who used this method were able to demonstrate *STAT6* mutation in 40% of patients<sup>265</sup>, in concordance with other studies.

## Sarcomas

Sarcomas are rare and heterogeneous malignancies. Soft tissue extremities and retroperitoneal sarcomas, bone tumors, and GIST will be included in this recommendation.

When should a somatic panel test be requested and for which patients? When is the best time to request? What should be assessed in a somatic mutation panel (which genes should be included and what alterations are expected from each gene)? How? Which platforms or tests are the most appropriate?

1. Consider molecular tests for assessment of somatic alterations in patients whose morphology and IHC are not sufficient to establish an accurate diagnosis, or it is necessary prognostic/predictive information. The method to be considered can be guided according to the suspected diagnosis, and availability. Alternatively, broad sequencing of multiple genes using NGS may be considered (type of recommendation: evidence-based evidence; strength of recommendation: strong);
2. Consider NGS sequencing test for the diagnosis, prognosis and therapeutic approach for non-GIST sarcomas (type of recommendation: consensus-based; strength of recommendation: weak);
3. Consider mutational assessment in GIST (genotyping) when planning adjuvant treatment. There is no preferred method (type of recommendation: consensus-based; strength of recommendation: moderate);
4. Consider mutational assessment in GIST (*KIT*, *PDGFRA*, *NF1*, *RAS*, and *SDH*) when planning treatment for the metastatic or inoperable disease. There is no preferred method (type of recommendation: consensus-based; strength of recommendation: moderate);
5. If the initial treatment of GIST has not been guided by molecular assessment, *PDGFRA* D842V assessment must be done in patients with progression of disease or evidence of primary resistance to imatinib (type of recommendation: evidence-based; strength recommendation: strong);
6. *ALK* translocation in patients diagnosed with inflammatory myofibroblastic tumors may be performed to confirm the diagnosis and guide potential therapy with an *ALK* inhibitor. It occurs in approximately 50% of IMFT<sup>266</sup> (type of recommendation: evidence-based; strength of recommendation: weak
7. *NTRK* fusion assessment may be performed in patients who have progression of disease after the first line, regardless of histology. The assessment may be done primarily by sequencing, FISH or IHC (type of

recommendation: evidence-based; strength of recommendation: strong);

8. *CDK4* and *MDM2* amplification assessment may be performed for using *CDK4* inhibitors. Well-differentiated liposarcomas represent 97% of the total. However, only the undifferentiated ones present this alteration<sup>267</sup> (type of recommendation: evidence-based; strength of recommendation: weak).

## DISCUSSION

The somatic mutations identified in most sarcomas are not driver mutations and, consequently, their assessment will result in limited immediate clinical benefit to the patient. On the other hand, the somatic mutations found at the time of diagnosis are important to detail the histological diagnosis and, subsequently, direct the best therapy. IHC, combined with the histology analyzed by a pathologist specialized in sarcomas, is an accessible method for the classification of the sarcomas. For a diagnostic definition, molecular tests are strongly recommended as auxiliary and complementary methods to IHC<sup>268</sup>. More than 30% of sarcomas have a known translocation<sup>269</sup>. Thus, the use of in situ hybridization (FISH), or even the real-time PCR method to detect these alterations, should be performed in patients whose histological diagnosis cannot be made with precision only by morphology or IHC<sup>270</sup>. An example of the importance of fusion assessment is the identification of several translocations in round cell tumors. *EWS* translocation is numerous and is increasingly recognized as a prognostic marker in Ewing's sarcomas and Ewing-like sarcomas<sup>270-272</sup>.

NGS with a large panel can identify numerous somatic alterations in sarcomas. However, the chance of finding any molecular alteration that is a target for treatment is low. This strategy may be used when there is the possibility of including the patient in clinical studies<sup>273</sup>.

In a study conducted at the MD Anderson Cancer Center with 102 patients with recurrent and metastatic sarcomas, the main alterations found were mutations in *TP53* (31%), *CDK4* (23%), *MDM2* (21%), *RB1* (18%), and *CDKN2A* (13%). Only 14/102 patients had a mutation that was the target of two approved drugs: pazopanib and imatinib. However, these drugs have an off-target effect in *PDGFRA*, *FGFR*, and *KIT*<sup>274</sup>. Therefore, broad sequencing may be used to aid in the histological classification and for the identification of patients for inclusion in clinical studies<sup>275</sup>.

Phase II studies evaluated the efficacy of *CDK4* inhibitors (palbociclib) in patients with well-differentiated and dedifferentiated liposarcoma with *MDM2/CDK4* amplification. The results show that this strategy results in disease control with promising progression-free survival, but with a low objective response rate<sup>276</sup>.

Crizotinib and ceritinib are *ALK* inhibitors that have shown activity in patients with inflammatory myofibroblastic tumors (IMFT), with *ALK* translocation<sup>277,278</sup>. Patients diagnosed with PEComa and lymphangi-

**Table 7.** Main somatic changes in Solid Hematologic Malignancies.

Type of cancer	Gene / Alteration	Test
Diffuse B-cell lymphoma	<i>CD10, BCL2, BCL6, MUM1, MYC, and BCL2</i>	IHC
Follicular lymphoma	<i>MYC, BCL2 e BCL6</i> translocation	FISH Currently, a specific genomic panel to assist in the evaluation of the evolution, therapeutic response, or risk of transformation for aggressive lymphoma is not recommended.
Hodgkin lymphoma	<i>Genomic panel is not recommended</i> <i>May be useful:</i> <i>LMP1 and PD-L1</i>	IHC for LMP1 assessment; it is a surrogate for the presence of EBV and PD-L1 expression assessment

oleiomyomatosis have been treated with mTOR inhibitors with promising results<sup>279</sup>.

On the other hand, patients diagnosed with GIST may have tumor genotyping performed at the time of diagnosis of the localized disease or at the time of the treatment of recurrent or metastatic disease<sup>280</sup>. The most frequent alterations in GIST are *KIT* and *PDGFRA* mutations. In approximately 15% of patients, no type of mutation was found in these two genes (*KIT* and wild *PDGFRA*). However, the wild type has been characterized by *NF1*, *BRAF*, *SDH1*, *RAS*, and *NTRK* mutations<sup>281-283</sup>. The presence of a mutation in exon 11 of the *KIT* gene is the most frequent and it is related to the increased sensitivity to imatinib in the setting of metastatic disease. Other mutations confer partial or total resistance to imatinib<sup>284</sup>. Prior knowledge of these mutations may better guide the therapeutic approach with alternative drugs, such as sunitinib, in the first mutation line in *KIT* exon 9<sup>285</sup>.

Recently, avapritinib, which potently inhibits the *PDGFRA* D842V286 mutation, was approved in the USA. Patients who develop secondary resistance to imatinib acquire new *KIT* or *PDGFRA* mutation, and the identification of the mutation may facilitate inclusion in clinical studies.

Molecular changes in *NTRK* genes are uncommon in adult sarcomas (0.76%)<sup>63</sup>. However, they can occur in more than 70% of patients in childhood fibrosarcoma, a rare disease that affects children, usually under one year of age<sup>287</sup>.

## Skin Cancer

### Melanoma

When should a somatic panel test be requested and for which patients? When is the best time to request?

1. The investigation of somatic mutations should be requested for every patient diagnosed with stages III or IV cutaneous, mucosal, or unknown primary melanoma (type of recommendation: evidence-based; strength of recommendation: strong). There is no recommendation, outside of research studies, for

carrying out tests that assess somatic mutations in stages I and II melanoma;

2. The assessment of somatic mutations should be requested at the time of diagnosis of melanoma (type of recommendation: evidence-based; strength of recommendation: strong).

What should be assessed in a somatic mutation panel (which genes should be included and what alterations are expected from each gene)?

3. For cutaneous, mucosal, or unknown primary melanoma, the presence of *BRAF* mutation should be assessed, with the most frequent mutation of type V600E and V600K (type of recommendation: evidence-based; strength of recommendation: strong).

4. For cutaneous (mainly melanoma of the subtype spitzoide), mucosa, or unknown primary melanoma, the presence of *NTRK* fusion should be assessed (type of recommendation: evidence-based; strength of recommendation: strong);

5. For cutaneous, mucosal, or unknown primary melanoma, the presence of *NRAS* mutation may be assessed. The most frequent is the Q61 type mutation, usually Q61L, and, less frequently, Q61R and Q61H (type of recommendation: informal consensus, recommendation strength: weak);

6. For mucosal or acral lentiginous melanoma, the *KIT* gene mutation may be assessed. The exons 9, 11, 13, and 17 mutations are the more frequent (type of recommendation: informal consensus; recommendation strength: weak).

How? Which platforms or tests are the most appropriate?

7. Several tests are available to assess the *BRAF* V600 mutation in melanoma, employing both DNA and antibody analysis. These tests are based on techniques such as real-time polymerase chain reaction (RT-PCR), mutation-specific or single gene, Sanger-type or NGS, pyrosequencing, high-resolution melting, and IHC, the latter using the antibody monoclonal



VE1. In Brazil, the most frequently used and accessible tests include commercially available versions that involve RT-PCR techniques (Cobas 4800, Idylla, THxID-BRAF) and that have high sensitivity and specificity for *BRAF* V600E and V600K mutation, however, they have low accuracy for other mutations in codon 600 (type of recommendation: evidence-based; strength of recommendation: strong). More recently, large genomic panels based on NGS and with different compositions have become available, including the main mutations of interest in melanoma (*BRAF*, *NRAS*, *KIT*, and *NTRK* fusions);

8. For assessment of *NTRK* fusion, IHC with pan-TRK antibody may be used as a screening test. However, it is necessary to have molecular confirmation of *NTRK1*, *NTRK2*, or *NTRK3* fusions, usually by NGS. Alternatives include fluorescent in situ hybridization (FISH) or RT-PCR (type of recommendation: evidence-based; strength of recommendation: strong);

9. The assessment of *KIT* and *NRAS* mutations is done by mutation-specific PCR or RT-PCR, Sanger-type sequencing, or NGS. It should be noted that the use of IHC to assess CD117 expression (c-KIT) is not validated for melanoma (type of recommendation: informal consensus; strength of recommendation: weak).

## DISCUSSION

*BRAF* mutation that constitutively activates the MAPK pathway is present in approximately 40 to 60% of melanomas. In 80 to 90% of patients, this activating mutation consists of the substitution of valine for glutamic acid at codon 600 (mutation V600E), and most of the others consist of an alternative substitution (valine for lysine) (V600K)<sup>288</sup>.

In the adjuvant setting, the phase III study (2) evaluated the use of the combination of dabrafenib plus trametinib versus placebo in 870 patients with a recent diagnosis of completely resected stage III cutaneous melanoma considered to be at high risk of recurrence (lymph node metastases > 1 mm), IIIB or IIIC, *BRAF* mutation (V600E or V600K). It was observed a statistically significant reduction of 51% in the risk of disease recurrence. After a median follow-up of five years, relapse-free survival was higher with dab-

rafenib plus trametinib (5-year rate, 52% vs. 36%; HR 0.51; 95% CI 0.42-0.61). After a median follow-up of 2.8 years, overall survival (OS) was longer with dabrafenib plus trametinib (3-year rate, 86% vs. 77%; HR 0.57; 95% CI 0.42-0.79). Based on this study, the combination of dabrafenib plus trametinib was approved for adjuvant use<sup>289</sup>.

In the metastatic scenario, *BRAF* and *MEK* inhibitors have also been to delay the development of resistance to treatment and to reduce some toxicities directly associated with *BRAF* inhibition<sup>290</sup>.

In the phase III COMBI-D study, 423 metastatic patients with *BRAF* V600E or V600K mutations, treatment-naïve, were randomized to receive dabrafenib plus trametinib, or dabrafenib plus placebo<sup>290</sup>. Progression-free survival (PFS) was significantly higher with the combination than with the use of dabrafenib alone (median 11.0 vs. 8.8 months, HR 0.67; 95% CI 0.53-0.84). The OS was higher with the combination (median 25.1 vs. 18.7 months, HR 0.71, 95% CI 0.55-0.92). With a minimum follow-up of 36 months, 19% of patients treated with the combination remained on therapy compared to 3% of those treated with dabrafenib alone<sup>291</sup>. The objective response rate (ORR) was significantly better (68% vs. 55%) with the combination compared to dabrafenib alone; CR rates were 18% against 15%, respectively.

A second phase III study, COMBI-V, randomized 704 patients with metastatic melanoma, *BRAF* mutation (V600) and not previously treated to receive dabrafenib plus trametinib, or vemurafenib as monotherapy<sup>292</sup>. OS increased significantly with the combination of dabrafenib plus trametinib (1-year survival: 72% vs. 65%, HR 0.69; 95% CI 0.53-0.89). The PFS at three years remained higher with the combination of dabrafenib and trametinib (25% vs. 11%); 58% of patients randomized to dabrafenib plus trametinib who were alive at age three remain on their original regimen. The median PFS also increased significantly (11.4 vs. 7.3 months, 95% CI 0.46-0.69), as well as the ORR (67% vs. 53%) in favor of the combination.

In a pooled analysis of the COMBI-D and COMBI-V studies, the combination of dabrafenib plus trametinib demonstrated median PFS and OS of approx-

**Table 8.** Main somatic alterations in sarcomas.

Cancer type	Gene / Alteration	Test
All adult sarcomas	<i>NTRK 1-3</i> (fusion) (0.76%)	IHC pan-TRK as a screening test for <i>NTRK 1-3</i> fusion; if positive confirm with NGS
Well-differentiated dedifferentiated liposarcoma	<i>CDK4</i> and <i>MDM2</i> (amplification) (>97%)	NGS
GIST	<i>KIT</i> , <i>PDGFRA</i> , <i>NF1</i> , <i>RAS</i> , and <i>SDH</i> (some of the alteration in > 98%)	NGS
Infant fibrosarcoma	<i>NTRK</i> (fusion) (>70%)	IHC pan-TRK as a screening test for <i>NTRK 1-3</i> fusion; if positive confirm with NGS
Inflammatory myofibroblastic tumor	<i>ALK</i> (fusion) (>50%)	RT-PCR ou NGS

imately 11 and 26 months, respectively<sup>293</sup>. The estimated PFS and OS in five years were approximately 19% and 34%, respectively. Among the 19% with a CR, the estimated OS in five years was 71%.

Another combination of BRAF and MEK inhibitors was tested using vemurafenib plus cobimetinib in a phase III study with 495 patients with previously untreated advanced melanoma and *BRAF* mutation that were randomized to vemurafenib plus cobimetinib, or vemurafenib plus placebo<sup>294</sup>. With a median follow-up of 14.2 months, PFS increased significantly with the combination of vemurafenib plus cobimetinib when compared to vemurafenib plus placebo (median 12.3 vs. 7.2 months, HR 0.58; 95% CI 0.46-0.72). ORR was increased with vemurafenib plus cobimetinib (70% vs. 50%), as well as the CR rate (16% vs. 11%). The median OS was also significantly longer with the combination (22.3 vs. 17.4 months, HR 0.70; 95% CI 0.55-0.90).

The third combination of BRAF and MEK inhibitors - encorafenib with binimetinib - was compared with the encorafenib or vemurafenib, both as monotherapy, in the COLUMBUS phase III study that included 577 patients with metastatic melanoma and *BRAF* (V600) mutation<sup>295</sup>. It was also observed a higher PFS with the combination, in comparison to vemurafenib (median 14.9 vs. 7.3 months, HR 0.51; 95% CI 0.39-0.67) and with encorafenib (median 14.9 vs. 9.6 months, HR 0.77; 95% CI 0.59-1.00). The OS was also superior with the combination than with vemurafenib alone (median 33.6 vs. 16.9 months, HR 0.61; 95% CI 0.47-0.79) or encorafenib (median 33.6 vs. 23.5 months, HR 0.81; 95% CI 0.61-1.06).

It is important to note that the three different combinations were not compared to each other in a phase III study.

In addition to *BRAF* mutation, the MAPK pathway can also lead to the development of tumors due to *NRAS* mutation. Binimetinib is a MEK inhibitor that has been studied particularly in patients with *NRAS* mutation. A phase III study randomized 402 patients with advanced melanoma and *NRAS* mutation to receive binimetinib or dacarbazine<sup>296</sup>. PFS was prolonged with binimetinib in comparison to dacarbazine (2.8 vs. 1.5 months, HR 0.62; 95% CI 0.47-0.80). ORR also increased with binimetinib (15% vs. 7%). However, there was no significant difference in OS (11 vs. 10 months) in a pre-specified interim analysis. Approximately 45% of the patients underwent subsequent immunotherapy, which may have masked a difference in OS.

KIT mutation is seen in approximately 15 to 20% of patients with acral or mucosal melanomas, and in a smaller percentage of melanomas that arise in areas of chronic skin damage. Phase II studies using imatinib or nilotinib in patients with advanced melanoma have shown only minimal activity<sup>297</sup>.

*NTRK* rearrangements can be found in a small subset of cutaneous and mucosal melanomas. Recently, the *NTRK* inhibitor, larotrectinib, demonstrated an

objective response rate of 78% in tumors with *NTRK* family fusions, independent of the histology<sup>298</sup>.

## Central Nervous System Tumors

### Adult gliomas

When should a somatic panel test be requested and for which patients? When is the best time to request?

1. The assessment of somatic mutations should be requested for all patients diagnosed with diffuse glioma (type of recommendation: evidence-based; strength of recommendation: strong);
2. The assessment of somatic mutations should be requested preferably at the time of diagnosis (type of recommendation: evidence-based; strength of recommendation: strong).

What should be assessed in a somatic mutation panel (which genes should be included and what alterations are expected from each gene)?

3. The presence of the *IDH* mutation should be investigated for diffuse gliomas in adults (astrocytomas, oligodendrogliomas, and glioblastomas) at the time of diagnosis (type of recommendation: evidence-based; strength of recommendation: strong);
4. The complete loss of chromosomal arms 1p and 19q (codeletion of 1p/19q) should be assessed in adult patients diagnosed with diffuse glioma and oligodendroglial phenotype who have *IDH* mutation (type of recommendation: evidence-based; strength recommendation: strong);
5. In diffuse gliomas with astrocytic histology, loss of *ATRX* and *TP53* mutation, codeletion assessment is not recommended (recommendation type: evidence-based; recommendation strength: strong);
6. The presence of the K27M mutation in the gene of the H3 family of histone 3A (*H3F3A*) should be assessed in all adult patients diagnosed with diffuse midline glioma (spinal cord, thalamus, brainstem, and cerebellum) (type of recommendation: evidence-based, strength of recommendation: strong);
7. The presence of *NTRK* fusion should be assessed in patients diagnosed with diffuse glioma (type of recommendation: evidence-based; strength of recommendation: strong);
8. The assessment of *BRAF* V600E mutation in selected patients; in adults, mainly in pleomorphic xanthoastrocytoma (type of recommendation: evidence-based; strength of recommendation: strong);
9. The assessment of *EGFR* amplification, chromosome 7 gain, chromosome 10 loss, and presence of the *TERT* promoter mutation may be considered in patients with diffuse astrocytoma and wild *IDH* (type of recommendation: formal consensus; strength of recommendation: moderate);
10. The assessment of *ATRX* and *TP53* mutations, in addition to the assessment of homozygous deletion in *CDKN2A* and/or *CDKN2B*, may be considered in

patients diagnosed with diffuse glioma with *IDH* mutation (type of recommendation: formal consensus; strength of recommendation: moderate).

How? Which platforms or tests are the most appropriate?

11. *IDH* mutation: IHC for *IDH1* mutation with R132H antibody is considered the preferred method. Negative cases should always be selected for sequencing (type of recommendation: evidence-based; strength of recommendation: strong);

12. Codeletion 1p/19q: FISH is the method of choice for evaluating codeletion 1p/19q in patients diagnosed with diffuse glioma and *IDH* mutation, although there is no consensus about what the gold-standard is. If the method is not available, the IHC indicating *ATRX* mutation (loss of *ATRX* nuclear expression) is considered characteristic of astrocytomas and mutually excluding with the presence of codeletion 1p19q. Other methods may be used as an alternative (e.g. CISH, PCR-based microsatellite analysis, RT-PCR, MLPA, and SNP array). The ideal test should identify the partial or complete loss of the chromosomal arms (type of recommendation: evidence-based; strength of the recommendation: strong);

13. *H3K27M* mutation: IHC is the preferred method in patients diagnosed with diffuse midline glioma. Sequencing methods may be used as an alternative (type of recommendation: evidence-based; strength of recommendation: strong);

14. *NTRK* fusions: sequencing methods that identify gene fusions are the method of choice for identifying these alterations in CNS tumors. IHC is not adequate in this scenario because it has a high rate of false positivity (type of recommendation: evidence-based; strength of the recommendation: strong);

15. *EGFR* amplification, gain of chromosome 7, loss of chromosome 10, presence of *TERT* promoter mutation, and assessment of homozygous *CDKN2A* and/or *CDKN2B* deletion: preferably requested within a wide sequencing panel. Alternatively, FISH or high-resolution cytogenetic methods (e.g., array-CGH, SNP arrays, and methylation arrays) may be used to detect homozygous *CDKN2A/CDKN2B* deletions and chromosomal losses (type of recommendation: formal consensus; strength of recommendation: intermediate);

16. *ATRX* and *TP53* mutations: may be assessed by IHC, mainly for diagnosis. Alternatively, they can be evaluated on a sequencing panel (type of recom-

mendation: evidence-based; strength of the recommendation: strong);

17. *BRAF* V600E mutation: preferably, assessment in a gene sequencing panel is recommended. Alternatively, it can be assessed by IHC (type of recommendation: formal consensus; strength of the recommendation: strong);

18. Assessment of somatic alterations in circulating tumor DNA (liquid biopsy) should not be considered in CNS tumors (type of recommendation: formal consensus; strength of recommendation: intermediate).

## DISCUSSION

Molecular tests were incorporated into the Classification of Primary CNS Tumors, according to the World Health Organization (WHO), in 2016<sup>299</sup>. Therefore, they must be requested at the time of diagnosis. In addition, the majority also have an important prognostic role. Type 1 or 2 isocitrate dehydrogenase (*IDH*) mutations have been identified as early events in the development of diffuse gliomas. They are present in all oligodendroglial tumors and are more common in patients diagnosed with WHO diffuse grade II glioma (59-90% of patients) when compared to grade III gliomas (28-82%) and grade IV gliomas (10 %). Its presence provides a better prognosis for tumors with wild *IDH*. When seen in high-grade glioma, they suggest that the tumor developed from a low-grade precursor lesion. About 80-90% of cases are identified by IHC, which can also help to differentiate reactive gliosis from tumor infiltration<sup>300</sup>. Gene sequencing can be used in cases with negative immune tests. The development of *IDH*-inhibitor therapy represents a promising strategy and may confer a predictive role to the marker<sup>301</sup>.

For the diagnosis of oligodendroglioma, the presence of the *IDH1/2* mutation associated with codeletion 1p/19q is necessary. This is caused by an unbalanced translocation between chromosomes 19 and 1, with total loss of a hybrid chromosome (1p;19q) and loss of heterozygosity. One of the most practical tests for detecting the 1p/19q codeletion is FISH (fluorescence in situ hybridization), although it may result false positive in partial or incomplete deletions. The 1p/19q codeletion is widely recognized as a prognostic and predictive marker, associated with prolonged patient survival, in addition to a better response to chemotherapy<sup>302</sup>. Almost all oligodendroglial tumors, with 1p/19q codeletion and *IDH*

**Table 9.** Main somatic alterations in melanoma.

Type of Melanoma	Gene / Alteration	Test	Comments
All	<i>NTRK 1-3</i> (fusion) (0.8%) <i>BRAF</i> (40-60%) <i>NRAS</i> (15-20%) <i>KIT</i> (10-15%)	IHC pan-TRK as a screening test for <i>NTRK</i> 1-3 fusion; if positive, confirm with NGS  NGS for the other alterations	Assessment of <i>KIT</i> mutations in acral or mucosal melanoma

mutation, have activating mutations in the promoter region of the telomerase reverse transcriptase (*TERT*) gene, however, it is worth remembering that these mutations are also frequent in wild *IDH* glioblastomas, conferring a worse prognosis in this scenario<sup>303</sup>.

The *TP53* mutation is identified in 36-60% of adult gliomas. Loss of *ATRX* expression is strongly associated with *IDH1/2* mutations and was identified in 65-97% of astrocytomas with *IDH1/2* mutation. Its agreement with *TP53* mutations occurs in 70-94% of cases. It is important to note that the *ATRX* and *TP53* mutations are almost mutually exclusive of the presence of 1p/19q codeletion, therefore, their identification can be used as a screening method<sup>304</sup>.

The diffuse midline glioma with H3K27M mutation is identified as a subgroup with K27M mutation in the histone H3 family gene 3A (H3F3A), or in the H3 histone family gene in cluster 1/B (*HIST1H3B/C*). It presents a glial phenotype and a diffuse growth pattern, in addition to being located in the midline. Morphology and molecular changes are important for its definition since H3K27M mutations are not exclusive to midline gliomas. This tumor occurs predominantly in young patients, located in the midline (spinal cord, thalamus, brainstem, and cerebellum), and has a worse prognosis, with a two-year survival rate below 10% and a median survival of nine months. The presence of the H3K27M mutation can be demonstrated reliably using IHC<sup>305</sup>.

The understanding of how molecular changes affect the typing and classification of CNS tumors continues to evolve. In addition to the previously mentioned mandatory markers for diagnosis, updates proposed by the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy (cIMPACT-NOW consortium) suggest other changes with a potential prognostic impact. According to the proposal, a diffuse wild *IDH* astrocytoma, with *TERT* promoter mutation and/or amplification of the epidermal growth factor receptor (*EGFR*) gene and/or combined gain of chromosome 7, associated with the loss of chromosome 10, would be classified as a grade IV tumor by the WHO. The survival of these patients is similar to that of patients with glioblastoma (with classic histological findings), wild *IDH*, WHO grade IV<sup>306</sup>.

Likewise, anaplastic astrocytomas (WHO grade III) with *IDH* mutation should be tested for homozygous *CDKN2A/B* deletion and, if present, the tumor should be designated as grade IV. Mitotic activity remains a grading criterion. Microvascular proliferation and necrosis remain criteria for diagnosing grade IV tumors, although these tumors may behave less aggressively, particularly if they do not have a homozygous *CDKN2A/B* deletion. In neuropathological practice, FISH or high-resolution cytogenetic methods (e.g., array-CGH, SNP arrays, and

methylation arrays) may be used to detect homozygous *CDKN2A/B* deletions<sup>303</sup>.

*BRAF* alterations may characterize subtypes of gliomas. In adults, *BRAF* V600E point mutations are present in up to two-thirds of pleomorphic xanthoastrocytomas. Occasionally, diffuse gliomas may also present this alteration, with predictive potential for targeted therapy. A specific antibody is available for detection of the V600E mutation by IHC, but it may also be detected by sequencing<sup>307</sup>.

The TRK family of transmembrane receptors is composed of three proteins, TRKA, TRKB, and TRKC, which are coded, respectively, by the genes *NTRK1*, *NTRK2*, and *NTRK3*. These receptors play a crucial role in the development of the nervous system during embryogenesis and remain expressed in neuronal tissue after birth. Pathogenic fusions involving the *NTRK* genes result in oncoproteins. It is a rare agnostic alteration, but potentially present in any tumor type. In primary CNS tumors, the reported frequency is <1%, but it may be higher in pediatric gliomas<sup>308</sup>. The development of targeted therapy with TRK-inhibitor agents has made it mandatory to assess these alterations in all tumors. Specifically in tissue from CNS tumors, IHC, although available, is inadequate due to the high rate of false-positive results<sup>309,310</sup>.

Current neuro-oncology practice is increasingly dependent on the molecular diagnosis of tumor tissue. The classification of tumors according to histological findings, integrated with molecular findings, has a diagnostic, prognostic, and potentially therapeutic role.

#### 4. FINAL CONSIDERATIONS IN THE INTERPRETATION OF SOMATIC MUTATIONS AND CLINICAL ACTIONABILITY

In the last decade, somatic panels were quickly adopted for the identification of genomic alterations that could assist in the decision making regarding the selection of targeted therapies and patient management<sup>10,311-315</sup>. Currently, hundreds of laboratories around the world provide results of genomic tests based on somatic panels, generating tens of thousands of reports each year. However, there is still little uniformity as to the mechanisms of analysis of variants and standardization of sequencing reports<sup>316-318</sup>.

Despite the development of recommendations for validating NGS<sup>319</sup> tests, many challenges remain in the detection of somatic mutations. Among these challenges is the detection of subclonal or variant in low-purity tumor samples, as well as the distinction of changes in the germline or artifacts related to amplification or sequencing during the polymerase chain reaction (PCR). Direct comparisons of NGS tests developed in laboratories that use different sample processing and sequencing techniques demonstrate disagreement in results, which raises concerns regarding the accuracy of such tests<sup>320-322</sup>.

A recent analysis of the *in silico* algorithms, most commonly used to call mutation, revealed that the existing methods for the detection of somatic mutation can be influenced by factors that generate false-positive and false-negative results<sup>323</sup>. Computational approaches that implement machine learning for direct analysis of raw data from massive parallel sequencing may be useful in minimizing the amount of false-positive calls, optimizing the sensitivity for detecting real alterations in the tumor<sup>323</sup>.

The variants final annotation clinically relevant requires validated computational support for the accurate interpretation of actionable mutations of a given neoplasia<sup>324,325</sup>. On the other hand, it is known that manual curation is an essential part of the process of generating reports and of the public databases of somatic mutations<sup>326,327</sup>. The standardization of the curation process can improve quality control and interoperability between the available databases, facilitating the regulatory approval of these efforts for the clinical interpretation of variants<sup>20,328</sup>. In recent years, two important databases have stood out as public tools for curating somatic variants in cancer: OncoKB and CIViC, developed, respectively, by the Memorial Sloan Kettering Cancer Center<sup>329</sup>, and the Washington University School of Medicine<sup>330</sup>.

The OncoKB (Precision Oncology Knowledge Base) database includes biological, clinical and therapeutic information, curated by resources from unstructured information, including recommendations and guidelines from the FDA (Food and Drug Administration), NCCN (National Comprehensive Cancer Network), and expert groups<sup>329</sup>. Since the clinical implications vary substantially based on the specific alteration in a given gene and the context of the tumor, the in-

formation in OncoKB is hierarchically organized by gene, alteration, type of cancer and potential clinical implications. OncoKB information is publicly available through an interactive website (<http://oncokb.org/>) and incorporated into cBioPortal for Cancer Genomics (<http://cbioportal.org/>)<sup>331,332</sup>, facilitating the interpretation of complex genomic data for oncologists and cancer investigators. To date, OncoKB has noted more than 5,300 changes in 682 cancer-associated genes in 55 types of cancer.

The CIViC database (Clinical Interpretation of Variants in Cancer) currently contains 7,532 cured interpretations of clinical relevance for 2,622 variants that affect 431 genes (<https://civicdb.org/home>). These interpretations were selected from 2,737 studies published by 256 CIViC curators<sup>330</sup>. CIViC evidence records are supported by a wide range of levels of evidence, currently focused on somatic alterations and positive associations with response to treatment. At least one evidence record has been created for 309 cancer subtypes and 454 drugs, with most data available for gene actionability in the lung, breast, colorectal cancer, and hematological tumors.

Since the public release of CIViC in June 2015, external curators (not affiliated with the Washington University) have contributed with almost half of all evidence records in this database, indicating the importance of external longitudinal collaborations in curating somatic variants. Like OncoKB, the CIViC database can be accessed free of charge without the need for registration or login. Both academic and commercial adoption of these databases should be widely encouraged by oncologists, oncology surgeons, radio-oncologists, pathologists, and investigators in general.

**Table 10.** Main somatic alterations in gliomas.

Gene / Alterations	Test	Comments
<i>NTRK 1-3</i> (fusion) (<1%)	IHC pan-TRK as a screening test for <i>NTRK 1-3</i> fusion, if positive confirm with NGS	
1p/19q (codeletion) (oligodendrogliomas only)	FISH	
<i>IDH</i> (up to 90% of grade II glioma)	NGS	Mutations in the gene of the H3 family of histone 3A (H3F3A) are present in midline tumors Mutations in the TERT promoter region are present in diffuse astrocytoma and wild IDH gliomas
<i>ATRX</i>		
<i>TP53</i> (36-60%)		
<i>H3K27M</i> (midline gliomas)		
<i>BRAF</i> (V600E) (1-5% diffuse gliomas in adults)		
<i>EGFR</i>		
Cytogenetic changes: Chromosome 7 gain, chromosome 10 loss, and presence of TERT promoter mutation	Comparative genomic hybridization (CGH-array)	

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