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HEALTH SCIENCES

Gliomas molecular markers: importance in treatment, prognosis and applicability in brazilian health system

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Abstract: Gliomas represent 80% of all primary malignant brain tumors in adults. In view of this public health problem, the early detection through sensitive and specific molecular tumor markers analysis can help to improve gliomas diagnosis and prognosis as well as their staging, assessment of therapeutic response and detection of recurrence. Therefore, this review focuses in current gliomas tumor markers, IDH-1/2, 1p/19q, MGMT, ATRX, TERT, H3, EGFR, BRAF and Ki67 used in clinic worldwide and their importance to early detection, glioma histological and molecular classification as well as in predicting patient's therapeutic response. In addition, we present what are the steps in the requesting process for this type of examination in the Brazilian Public Health System (SUS) scope, which attends most of the Brazilian population. Thereby, this article is useful in demonstrating which markers are used in the clinical practice for glioma patients and can be performed in the SUS through partnerships/agreements between specialized health centers and clinical analysis laboratories. It is hoped that this work clarifies, the necessary subsidies to carry out the research of tumor markers in all institutions that serve SUS users, providing a service with equal conditions.

Key words: SUS, tumor markers, cancer, glioma, diagnosis, public health.

INTRODUCTION

Cancer was the cause of approximately 10 million deaths worldwide in 2020 (Ferlay et al. 2020). For the year 2030, statistical projections of the World Health Organization (WHO) estimate 27 million incident cases, 17 million deaths and 75 million people alive, each year, with cancer (Grech et al. 2020). In Brazil, 625 thousand new cases are estimated for each year of the 2020/2022 triennium (INCA 2019). Due to its chronic and epidemic character, its morbidity and mortality continues to increase globally, causing great physical, emotional and financial losses to the affected patients, their families and communities, and to the health systems. The most affected by this disease are patients who do not have access to diagnosis and treatment in a timely manner, especially those assisted by public health systems in low- and middle-income countries (Martel et al. 2019).

The central nervous system (CNS) tumors are among the 10 largest global causes of death (Ostrom et al. 2020), being the estimated global incidence 8–10/100,000 population per year (Jokonya et al. 2021). Among the CNS tumors, the ones committing the brain cause about 2% of all cancer deaths (Jokonya et al. 2021). The most common primary malignant brain tumors are the gliomas, accounting for 80% of all diagnosed cases in adults (Fisher & Adamson 2021). Gliomas are heterogeneous macroglial cell malignancies (Chen et al. 2019) that derive from astrocytes,

oligodendrocytes, Schwann cells and ependymal cells and neural progenitors (Azzarelli et al. 2018) and extensively infiltrate the brain parenchyma (Chen et al. 2019). In the Brazilian scenario, the burden of both gliomas and other types of cancer is aggravated by two main points: (i) the gradual increase in cancer incidence and mortality, in proportion to demographic growth, population aging and socioeconomic development; and (ii) the challenge faced by the Brazilian Public Health System (SUS) in ensuring full, universal and equitable access for the population to the diagnosis and treatment of cancer (Alves et al. 2017). In this sense, the main obstacles in the path of the patient with glioma in view of the Brazilian Collective Health Plan (for review see: Brazil 2009) relate to the availability of services and the integration of actions at different levels of care, as well as the lack of information about the disease and the objective of carrying out preventive exams (Marques 2014). Therefore, it is worth asking: (i) what is the path taken by the patient with glioma treated at SUS, from the appearance of the first symptoms to the realization of the treatment? and (ii) what new technologies are being explored and leveraged to improve this service? Are such health-producing practices really capable of improving the diagnosis of patients and prognosis stages? Simply put, the oncology care network in SUS is constituted of health establishments qualified as a High Complexity Assistance Unit in Oncology (UNACON) or as a High Complexity Assistance Center in Oncology (CACON). In these establishments, patients with cancer must be covered with proper assistance regarding seven integrated modalities: diagnosis, support measures, palliative care, rehabilitation, oncological surgery, radiotherapy and chemotherapy (hematology and clinical and pediatric oncology) (Brazil 2018). As part of care performed by SUS in Brazil, the applicability of glioma molecular marker is a strategy for improving decision-making from diagnosis to prognosis of gliomas. It is possible through dosing methods of certain substances present in or produced by cells, which provide information about cancer, as a staging parameter, therapy control and prognostic factor (Santos et al. 2020, NCI 2021).

This review article focuses on truly implemented and accessible molecular biomarkers conveying glioma information, whose detectable alterations have direct implication on clinical decision-making process in standard of care (SOC) of Brazilian patients. It will be described the glioma molecular markers IDH-1/2, 1p/19q, MGMT, ATRX, TERT, H3, EGFR, BRAF and Ki67 due to their significance in the diagnostic and prognostic routine as well as their utility in clinical management practices worldwide. Among them, IDH-1/2, 1p/19q, MGMT, ATRX and Ki67 appear in the "Recommended Cancer Treatment Guidelines by the Brazilian Society of Clinical Oncology" (SBOC) and IDH-1/2, 1p/19q and MGMT appear in the "Joint Ordinance No. 7 of April 13, 2020", which approves the "Diagnostic and Therapeutic Guidelines for Brain Tumor in Adults" (Brazil 2020b), based on currently available clinical evidence in Brazilian context (Brazil 2020b) and in the recommendations of the 2016 WHO classification of tumors of the CNS system (Louis et al. 2016). A variety of other relevant glioma molecular markers has been reported, for instance NOTCH1, CIC, FUBP1, TP53 and PTEN, although, due to conflicting results and variability across the studies these markers have not been fully included neither in the 2016 WHO classification of gliomas nor Brazilian. However, since these biomarkers are undergoing constant investigation, they have been included in 2021 WHO classification (Louis et al. 2021) and they will certainly be gradually included in future Brazilian guidelines.

The present study performed exploratory research on articles and guidelines from the World Health Organization, American Cancer Society, José Gomes de Alencar National Cancer Institute and websites of the Ministry of Health of Brazil (available at https: //www.gov.br/health/pt-br), in addition to Brazilian norms (Laws, Decrees, Ordinances and Resolutions), Pubmed, Scielo and Science Direct databases. The choice of articles in the databases was based on the search for the descriptors "cancer", "cancers of the central nervous system", "glioma", "glioma therapy", "SUS", and "tumor markers". Secondly, the descriptors "IDH-1/2", "1p / 19q", "MGMT", "ATRX", "TERT", "H3", "EGFR", "BRAF" and "Ki67" were crossed with the words "mutations", "tumor marker", "gliomas" and "revision". Information was collected from articles published in the period 2007-2021, which met the previously defined objective of addressing the molecular markers of glioma, their importance in treatment, prognosis and applicability in the Brazilian Unified Health System. In this process, articles that had only abstracts were excluded, due to insufficient data for this review. As a final result of the search process, 82 references were reached, resulting in four major categories: (i) 12 on cancer, (ii) 23 on glioma, (iii) 9 on SUS and (iv) 38 on tumor markers. Finally, the major limitation of this work was the lack of information about the history of tumor markers in the SUS, as well as their inclusion and regulation process.

In addition, this article analyzes the possible paths of the patients with glioma and the processes of clinical practice of health professionals who assist them in the scope of SUS. In this sense, glioma has been described, from diagnosis to therapy. It demonstrated the steps that the patient goes through when he/she is assisted within the SUS model, as well as the importance of combining the technology of tumor markers in clinical practice and public health policies in the Brazilian context. The tumor markers presented in this study can guide SUS managers in the implantation, monitoring and evaluation of results arising from its use. Therefore, with this research, it is stated that it is possible to build a valuable clinical practice that combines continuous education of health professionals, the use of diagnostic, therapeutic and prognostic tools offered by SUS and the incorporation of aggregating elements such as the testing of tumor markers. All of this by engaging in a collective and moving plan, where these clinical practices become political, which strengthen the entire ideology of a universal, integral and equitable SUS.

GLIOMAS

Gliomas are characterized as tumors of rapid and progressive growth, low incidence rates, but high mortality rates (Molinaro et al. 2019), both with an increasing trend in the coming years, especially in developing countries (Grech et al. 2020). The process of diagnosing glioma begins when the patient seeking a health service undergoes an initial assessment consisting of detailed physical examination and neuroimaging tests (Brazil 2020b, Santos et al. 2020, Heemann & Heemann 2018). In case of suspected glioma, traditionally, the only imaging test recommended for diagnosis and initial evaluation is magnetic resonance imaging of the skull. Other tests, such as computed tomography and spectroscopy/perfusion, are necessary if secondary lesions are suspected (SBOC 2021, Santos et al. 2020, Heemann & Heemann 2018). Then, the investigation with biopsy and histopathological analysis follows (Brazil 2020b, Santos et al. 2020). At this stage of investigation of the tumor, it is also possible to count on the help of tumor marker exams, which are facilitating components in the elaboration

of strategies that seek to better understand the characteristics of cancer at the same time that they provide diagnostic, prognostic and/or predictive applicability (Santos et al. 2020).

In Brazil, currently, the histopathological classification of gliomas follows the 2016 WHO classification of tumors of the CNS recommendations, initially published in 1979 and revised five times since then, the most recent in 2021, as well as recommendations of the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy (cIMPACT-NOW) (Louis et al. 2016). Therefore, it appears in the Cancer Treatment Guidelines recommended by the Brazilian Society of Clinical Oncology that molecular analysis is mandatory to establish the correct diagnosis of glioma (SBOC 2021). Thus, histology and immunohistochemistry parameters associated with advances in molecular biology, such as tumor markers testing and tumor phenotypic characteristics understanding, provided a better prognostic prediction and more appropriate therapeutic targeting of tumors (Santos et al. 2020, Louis et al. 2021).

Gliomas according to the recommendations of the 2016 WHO classification

In 2016, WHO used current molecular parameters combined with histological parameters to classify newly recognized "entities", "variants" and "tumor patterns", and to eliminate some already described that no longer have a diagnosis and/or biological relevance. Therefore, a way of naming these tumors with more precision was formulated and a new way of diagnosing them was structured (Louis et al. 2016). Thus, there has been an "integrated diagnosis", which comprises (i) neuroimaging tests; (ii) histological classification; (iii) WHO degree; and (iv) molecular information (Figure 1) (Louis et al. 2016, Kristensen et al. 2019).

The 2016 WHO classification restructured diffuse gliomas, medulloblastomas and other embryonic tumors. It also reorganized glioblastoma (GBM) according to its mutation profile in the IDH gene. Further, the classification included brain invasion in the criteria for atypical meningioma. Additionally, it reclassified the solitary fibrous tumor/hemangiopericytoma tumors as a single entity (Louis et al. 2016). Since 2015, the improvement in the classification of tumors was due to the addition of immunogen type parameters through immunohistochemistry and molecular markers (Louis et al. 2016). In the case of gliomas, they are: IDH-1/2, 1p/19q, MGMT, ATRX, TERT, H3, EGFR, BRAF and Ki67 (Louis et al. 2016, Kristensen et al. 2019).

With regard to the nomenclature of tumors, an association between the morphological diagnosis and its molecular signature was proposed (Louis et al. 2016). The WHO classification included the nomenclatures: diffuse astrocytoma (IDH-mutant and non-mutant) and anaplastic (IDH-mutant and non-mutant); diffuse gliomas, including grade II and III astrocytic gliomas; grade II and III oligodendrogliomas; grade IV GBM; diffuse childhood-related glioma and diffuse midline glioma; ependymoma H3 K27M-mutant; medulloblastoma WNT-activated and SHH-activated; and embryonic tumor with multiple layers of rosettes, altered C19MC (Louis et al. 2016). In addition, there was the inclusion of a provisional category, classified as "Not Specified Elsewhere". It was used when the tumors did not have a characteristic molecular profile or it was not possible to perform the search for these genotypic changes, due to the unavailability of the analysis by immunohistochemical study or the unavailability to perform the sequencing of the tumor's genetic material (Louis et al. 2016, Reifenberger et al. 2017). Classically, tumors can be classified based on the type of glial

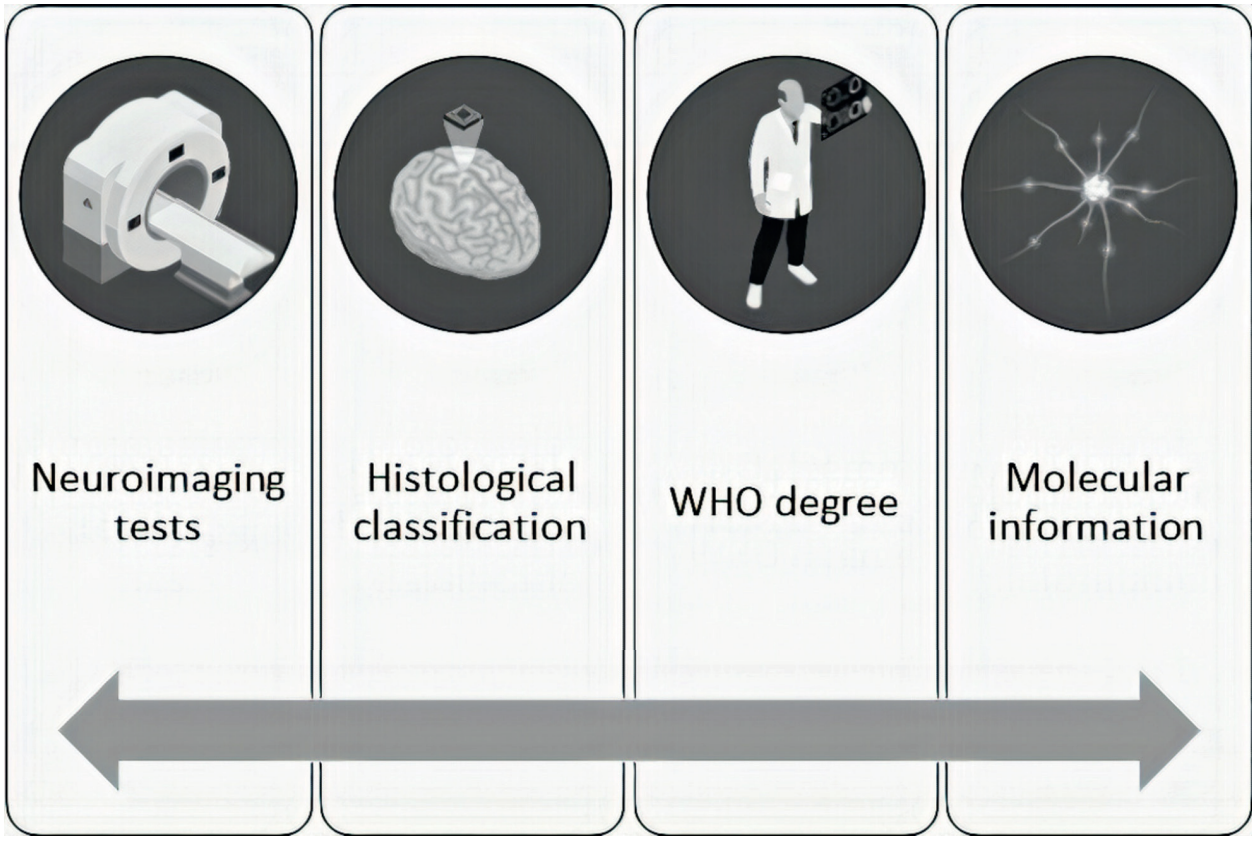

Figure 1. Diagnosis of gliomas. The "integrated diagnosis" workflow used in gliomas diagnostics comprises (i) neuroimaging tests; (ii) histological classification; (iii) WHO degree; and (iv) molecular information.

cell of origin: astrocytoma, oligodendroglioma, ependymoma and schwannoma. There are also "mosaic" tumors, which have characteristics of both astrocytoma and oligodendroglioma, being called oligoastrocytoma. In addition to the morphological differences, there is a subtype classification according to the similarities and peculiarities of the tumors, which involves genetic aspects, malignancy, tumor development, therapeutic sensitivity and the prognosis of the patient (for review see: Louis et al. 2007).

Concerning tumor malignancy, it was assessed on a scale ranging from I to IV. For histological aspects, low grade I and II gliomas were slow-growing, differentiated and subject to surgical resection, and grade I tumors were typically found in children (for review see: Louis et al. 2007). Among the low-grade gliomas, the 5 main subtypes were: diffuse astrocytoma (mainly in adults aged 30 to 40), pilocytic astrocytoma (almost exclusively in individuals under 25), common oligodendroglioma (the second most common type low-grade glioma), gangliogliomas (a mixture of pilocytic astrocytoma and neuronal cells; individuals in their 20s), mixed gliomas (usually presenting characteristics of diffuse astrocytoma and oligodendroglioma) (for review see: Louis et al. 2007).

Finally, the classification was made based on the location of the tumor, which was based on a membranous structure that separates the brain from the cerebellum, the cerebellar tentacle (or cerebellar "roof"). Thus, gliomas could be defined as supratentorial, which develop above the tentacle (in the brain) and correspond to 70% of the gliomas in adult patients, and

infratentorial, which develop below the tentacle (in the cerebellum) and correspond to 70% of the gliomas in pediatric patients (for review see: Louis et al. 2007).

Gliomas according to the recommendations of the 2021 CNS WHO classification

The 2021 Brazilian classification of CNS tumors was published previously to the release of the WHO CNS 2021 classification update (Brazil 2020b, SBOC 2021). For this reason, the new WHO recommendations have not yet been implemented in Brazil, nevertheless they will be briefly described in this review as they will certainly be gradually included in future Brazilian guidelines. In 2021, the fifth edition of the WHO CNS classification addresses the inclusion of the DNA methylome profile of CNS tumors as a promising and reliable auxiliary diagnostic tool, as each tumor has its own methylation signature (Louis et al. 2021). Although it can be used when available, there are reservations about the best methodology to be used, as well as regulatory issues for each DNA methylome profile, which limits this technique to be more widely available. As regards its applicability, the recommendations in the sections "Definitions and Essential and Desirable Diagnosis Criteria" should be followed (for review see: Louis et al. 2021). Importantly, it is emphasized in the 2021 WHO new classification of CNS tumors that the taxonomy described represents an intermediate stage, understood as a transition stage, for a future even more precise classification (Louis et al. 2021).

The new WHO classification *de novo* restructures diffuse gliomas, medulloblastomas, embryonic tumors, among others. Further, it includes MAPK pathway alterations; histological and histogenetic similarities; as well as molecular features to reclassify tumors. Remarkably, the sixth WHO classification standardizes the terms "type" and "subtype" instead of "entity" and "variant", respectively (Louis et al. 2021). Regards gliomas, glioneuronal tumors and neuronal tumors, the new classifier approach divides them into 6 groups: (i) adult-type diffuse gliomas, (ii) pediatric-type diffuse low-grade gliomas; (iii) pediatric-type diffuse high-grade gliomas; (iv) circumscribed astrocytic gliomas; (v) glioneuronal and neuronal tumors; and (iv) ependymomas (Louis et al. 2021). It is highlighted that the 2016 WHO previously tumors named "diffuse astrocytoma", "anaplastic astrocytoma" and "GBM" are currently named, within 2021 WHO parameters, as a single tumor type, "astrocytoma, IDH-mutant". Additionally, they are now graded as "CNS WHO grades 2, 3 and 4". Besides, it was incorporated genetic parameters for a diagnosis of GBM, IDH-wild-type in adults, if there is microvascular proliferation or necrosis or TERT promoter mutation or EGFR gene amplification or +7/–10 chromosome copy number changes (for review see: Louis et al. 2021). Lastly, the nomination "astrocytoma, IDH-mutant" covers grades 2-4 and eliminates the nomination "GBM, IDH-mutant" and "diffuse midline glioma, H3 K27-mutant" is modified to "diffuse midline glioma, H3 K27-altered" (Louis et al. 2021). Other molecular parameters include astrocytoma IDH-mutant with CDKN2A/B homozygous deletion and diffuse astrocytoma IDH-wild-type with +7/–10 copy number changes, which allows, from then on, that a molecular parameter may, eventually, have value among the histological findings for the grading process of tumors (Louis et al. 2021).

With regard to the nomenclature of tumors, the recommendations of the 2019 cIMPACT-NOW Utrecht meeting are followed to make it simpler and more consistent (Louis et al. 2021). It has been proposed to use only genetic modifiers with clinical utility, location and age, except in some cases where morphological features are retained

in the name, because they are not prominent in all types of a specific group for example, "myxopapillary ependymomas", some of which are minimally myxoid while others may not be overtly papillary (Louis et al. 2021). In addition, some nomenclatures referring to histological features rooted in common usage were retained so as not to cause confusion in clinical practice, as well as in clinical and epidemiological studies, which draw on previously collected data, for example, "medulloblastoma". Furthermore, the modifier term "anaplastic" is no longer used in the new classification (for review see: Louis et al. 2021). Finally, the new 2021 WHO classification included the category "Not Elsewhere Classified", indicated by the suffix "NEC". It is used when diagnostic testing was performed, but the result does not allow a tumor classification within WHO criteria, due to incompatibility of histological, molecular and/or clinical features. In this case, pathologists categorize the tumor into a diagnosis that does not belong/is not conform to WHO standards (Louis et al. 2021). Regarding to gene names and gene symbols, the 2021 WHO classification utilizes the HUGO Gene Nomenclature Committee (HGNC) system (available in: https://www.genenames.org/) and, for sequence variants, it follows the recommendations of the Human Genome Variation Society (HGVS) (http://varnomen.hgvs.org/) as well as the reporting guidelines of the International System for Human Cytogenetic Nomenclature 2020 for chromosomal alterations (for review see Louis et al. 2021).

Concerning tumor grading, it is assessed closer to non-CNS tumors grading and, as previously cited for tumor nomenclature, it retaining some key parameters of classical CNS tumor grading, although two main parameters of CNS tumor grading have changed for new 2021 WHO classification: (i) Arabic numerals are employed and (ii) tumors are graded within "types". Notably, it is endorsed the use of the term "CNS WHO grade" instead of "WHO grade" to indicate the grading. Moreover, since CNS tumor grading has been used for decades as well as associated with expected clinical-biological behaviors, the sixth version of WHO classification has decided to maintain the grading parameters of prior editions. Importantly, currently, astrocytoma IDH-mutant is classified from 2-4 within CNS WHO grade and meningioma from 1-3 (for review see: Louis et al. 2021).

GLOBAL AND BRAZILIAN SCENARIOS OF GLIOMAS

Directing information about Brazil, National Cancer Institute José Alencar Gomes da Silva (INCA) assists in the planning and organization of actions to fight cancer. One of his most important contributions is the publication, every two years, of cancer references. The information is based on the various Hospital Cancer Registries and Population Based Cancer Registries existing in the country (INCA 2019). However, for the first time, a triennial estimate was carried out, which is expected to register 625 thousand new cases of cancer in each year 2020/2022 (INCA 2019). The INCA attributed the fact that the publication of the cancer estimate is for the first time three years to the improvement of the quality of the information in the cancer records, the maintenance of the historical series and chronic profile of the cancer, which would not present changes in its profile for short periods (INCA 2020). Furthermore, the INCA estimates for each year of the 2020/2022 triennium, 11,100 incident cases of CNS tumors, with 5,870 in men and 5,230 in women. This value corresponds to an estimated risk of 5.61 new cases per 100 thousand men and 4.85 new cases per 100 thousand women (INCA 2019). Further, according to the INCA, these cancers can originate from the

sum of changes acquired over time, by genetic predisposition, or by exposure (INCA 2019). Among the known risk factors are exposure to ionizing radiation, deficiency of the immune system, environmental exposures (arsenic, lead and mercury), occupational exposures (workers in the petrochemical, rubber, plastic and graphic industries) and obesity (ACS 2019, INCA 2019).

In the Brazilian scenario, the publication "Estimate 2020: cancer treatment in Brazil" reports that approximately one third of new cancer cases in the country can be avoided by reducing or even eliminating environmental risk factors and those related to lifestyle, such as smoking, excessive exposure to the sun and physical inactivity (INCA 2019). This information is corroborated by another study, carried out by the partnership between the Faculty of Medicine of the University of São Paulo and Harvard University, in the United States. It carried out a qualification of 114 thousand cases of cancer in Brazil (27% of the total) and 63 thousand deaths from the disease (34% of the total) in the national territory. Altogether, these numbers could be avoided per year by reducing five risk factors: smoking, alcohol consumption, overweight, unhealthy eating and lack of physical activity (Rezende et al. 2019).

GLIOMA THERAPY

The SOC for gliomas follows the Stupp protocol, consisting firstly of concurrent and adjuvant surgery (Fisher & Adamson 2021). Post-surgery is based on radiotherapy (RT), at a dose of 60 Gy in 30 fractions (usually administered for 6 weeks: 2 Gy per day, 5 days per week) (Fisher & Adamson 2021). Concomitantly, the cytostatic alkylating agent temozolomide (TMZ) is administered orally, at a dose of 75 mg/m^2 per day for the entire RT. RT is followed by TMZ 150 at 200 mg/m^2 for five days, every 28 days, for six months (Fisher & Adamson 2021). TMZ is easily absorbed orally, crosses the blood-brain barrier quickly and is better tolerated than other compounds of the triazene group, such as mitozolomide and dacarbazine (Strobel et al. 2019). It is a prodrug derived from imidazotetrazone that, when metabolized, generates the 5-[3-methyltriazene-1-yl]imidazole-4-carboxamide (MTIC) metabolite, responsible for its toxic effect, capable of forming the methyldiazonium ion, which methylates DNA at the N7 positions of guanine and 06 of methylguanine. Depending on the repair deficiency and the magnitude of the damage, it can lead to cell death in the G2/M phases, performing antitumor activity in a variety of cancers (Fisher & Adamson 2021, Johannessen & Bjerkvig 2012). In addition to TMZ, there are four drugs and one FDA-approved device for the treatment of high-grade gliomas: lomustine, intravenous carmustine, carmustine wafer implants, bevacizumab and tumor treatment fields (Fisher & Adamson 2021).

Management of glioma patients during SARS-Cov-2 pandemic

The SOC for gliomas has poorly increased the prognosis of patients with glioma, with 15 months of median survival and less than 5% of a 5-year survival rate (Huang et al. 2020). In addition, the worldwide health scenario that is no longer favorable for these patients, worsens and changes in the face of the pandemic due to severe acute respiratory syndrome generated by coronavirus 2 (SARS-Cov-2) (Batistella et al. 2021).

In the Brazilian scenario, the health institutions focused on adapting to use telemedicine when clinical indicated and on reducing local infection, providing high-grade glioma patients a safer environment. At the same time, a group of experts published some recommendations to guide the decision-making

process of these patients, considering their consent, age, molecular tumor profile and performance (Batistella et al. 2021). These recommendations reinforce the maximum safe resection for glioma as a first-line approach, since its benefit in overall survival (OS) and quality of life. In addition, based on a recent study, they recommend low-radiation RT, using 40Gy/15 fractions of 2.67Gy in three weeks instead of 60Gy in six weeks, besides the use of TMZ or RT alone, especially for elderly and with low-performance status patients. Importantly, chemo-radiotherapy should be accompanied by regular blood tests and close attention to collateral effects and toxicities as well as take into consideration the O6-methylguanine-DNA methyltransferase (MGMT) promoter and IDH mutation profile of tumor (Batistella et al. 2021). For patients with positive SARS-Cov-2 tests, it is strongly recommended to postpone their surgery until de infection ceases while for negative tests patients, it is recommended hospital admission via a route without contact with other patients to prevent cross-infection, in addition to individual accommodation accompanied by a strict quarantine period (Batistella et al. 2021).

It is noteworthy that there were no significant changes in SOC protocols in glioma patients worldwide because, there is no consensus concerning standard measures (Weller & Preusser 2020, Oliveira et al. 2021). The point is that some comorbidities, as cardiovascular, pulmonary and immunological status, in addition to currently SARS-CoV-2 infection, become the major risk factors in these patients, which can contribute to worsening the course of the disease. Thus, these comorbidities gain attention and become priority in the discussion boards of neuro-oncology societies and hospital multidisciplinary teams, who must determine the best approach after analyzing each case individually (Weller & Preusser 2020, Oliveira et al. 2021).

Tumor markers

Cells release macromolecules of protein character into the blood and other body fluids in response to benign (noncancerous) conditions. When these macromolecules are produced at higher amounts by tumor cells, including gliomas, they may function as tumor markers (Muller Bark et al. 2020). Described as mostly proteins or pieces of proteins (cell surface antigens, cytoplasmic proteins, enzymes and hormones), they can be measured biochemically, immunohistochemically or genetically in the tumor, blood and other body fluids of patients with cancer (Konings et al. 2020, Sokoll & Chan 2020, NCI 2021). However, the ideal tumor marker capable of providing an early diagnosis of the neoplasia and its origin, establishing the extent of the disease, monitoring the therapeutic response, detecting early recurrence, being organ-site specific and having a short half-life has not been found yet (Sokoll & Chan 2020, NCI 2021). Regarding circulating tumor biomarkers, there are available literature about future applicability of plasma circulating tumor cells, cell-free tumors, circulating cell-free microRNAs, circulating tumor DNA and circulating extracellular vesicles for the diagnosis and monitoring of brain tumor (Linhares et al. 2020, Ali et al. 2021, Jelski & Mroczko 2021). Although these serum biomarkers have been reported to better identify and classify gliomas and provide prognostic value for these patients, none of them had sufficient sensitivity and specificity nor could they be associated with clinical outcome to serve as a diagnostic biomarker for gliomas (Linhares et al. 2020). Therefore, due to significant limitations, such as rapid tumor development and recurrence, disease and patient heterogeneity, non-standardization of

sample collection, methods of quantification and validation of results, as well as studies with a low number of patients (Linhares et al. 2020), in addition to the fact that none of them are ready for clinical implementation and the focus of this review is precisely on current molecular markers already used in the clinic, circulating markers will not be discussed (Ali et al. 2021, Jelski & Mroczko 2021). It is noteworthy that the biomarkers already used in the clinic are facilitating tools in the development of anti-glioma strategies, as they expand the understanding of tumor characteristics by acting as predictors and prognostic hallmarkers (Ludwig & Kornblum 2017). Moreover, molecular genetic features, such as IDH-1/2, 1p/19q, MGMT, ATRX, TERT, H3, EGFR, BRAF and Ki67 confer different diagnosis and prognosis for gliomas (Szopa et al. 2017) (Table I). It is important to emphasize that, although results with current tumor markers present promising data, the parameters sensitivity, specificity and accuracy will not be discussed in this review because there was a large variation in reported results. This is mainly due to the small sample size (>100 patients) studied, differences in acquisition protocols, as well as in the reference standards that were used (Szopa et al. 2017). In this context, according to National Comprehensive Cancer Network (NCCN) Guidelines® for Central Nervous System Cancers, from United States, biomarkers testing is justified by the necessity of knowing specific mutations of the tumors to better direct therapy and future target therapies under investigation, what can improve individualized patient response. It must also be considered that biomarker testing not only guides treatment decision-making, but also improves the accuracy of diagnosis in cancer patients (NCCN 2021). In this sense, by making available the technology for testing glioma biomarkers, as well as all types of cancer, in the SUS, which is the health system that assists most of Brazilian population, it allows certain mutations to be detected, better characterizing each patient tumor and guiding a more accurate treatment. Each of glioma molecular markers included in this work were chosen to show its role in screening the disease, better characterizing it through diagnosis, staging and prognosis, when applicable, besides suggesting therapeutic interventions by monitoring or predicting responses to them (Table I).

MGMT

Regarding the DNA damage repair enzyme MGMT, it became the standard test of care for elderly patients (Li et al. 2020). It was due to its epigenetic gene silencing by promoter methylation, leading to reduced DNA alkylation repair efficiency and increased response to TMZ therapy, what ultimately prolongs OS and progression-free survival (PFS) in patients with high-grade diffuse gliomas (Li et al. 2020, Pandith et al. 2020). The methylation of the MGMT promoter is highly frequent in WHO grade II low-grade gliomas (80%), while lower frequent in WHO grades III and IV malignant gliomas (35–45%) (Pandith et al. 2020). The most common test used to analyze MGMT in clinical practice is the DNA-based methylation-specific PCR, though, there are other analytical methods to access MGMT protein status, for instance, mRNA expression testing, real time PCR, methylation specific multiplex ligation-dependent probe amplification, methylation specific pyrosequencing and IHC (Siegal 2016). The presence of MGMT methylation is a valuable prognostic and predictive biomarker because it is associated with a better response to TMZ and a longer OS of the patient (Heemann & Heemann 2018). In relation to MGMT methylation and extent of resection (EOR), it is reported

Table I. Molecular markers and their clinical relevance in gliomas.

Tumor Marker	Detection Methods	Diagnostic, Prognostic or Both value	Use of Laboratory Test	Tumor Type of Occurancy	Clinical relevance	References
IDH-1/2 mutation [isocitrate dehydrogenase 1 (R132H/C/L/S/G)] [isocitrate dehydrogenase 2 (R172K/M/G/W)]	- IHC - Sanger sequencing - Pyrosequencing - NGS	- Both	- Recommended in all gliomas -Sequencing recommended if IDH1 R132H negative by IHC	- Low grade gliomas - WHO grade II and III astrocytomas - Oligodendrogliomas - Secondary GBM	- Molecular parameters for glioma classification - Presence of both variant and 1p/19q codeletion defines oligodendroglioma - Defines grade II and III astrocytomas, grade II and III oligodendrogliomas and secondary grade IV GBMs - Benefit from radiation or alkylating chemotherapy - Relatively favorable prognosis compared to IDH-wild-type astrocytic tumor with the histologically same WHO grade - Important in stratification for clinical trials - Associated with MGMT promoter methylation - Increased survival with alkylating chemotherapy or radiation -IDH1 R132H mutation may represent a promising target for mutation specific vaccination	Staedtke et al. 2016, Kristensen et al. 2019, Jiang et al. 2021, NCCN 2020.

Table I. Continuation.

1p/19q codeletion [short arm of chromosome 1 (1p)] [long arm of chromosome 19 (19q)]	- FISH - PCR - Array- or NGS-based methods	-Diagnostic	- Recommended in oligodendrogliomas	- Oligodendroglimoa - Anaplastic oligodendroglioma - Oligoastrocytoma - Anaplastic oligoastrocytoma	- Presence of both codeletion and IDH variant defines oligodendroglioma diagnosis - Diagnostic value enhanced by combined use of IDH-1/2, ATRX, CIC and FUBP1 - Predictive of response to alkylating chemotherapy and combination of radiation and alkylating chemotherapy - Relatively favorable prognosis	Staedtke et al. 2016, Kristensen et al. 2019, Jiang et al. 2021, NCCN 2020.
MGMT promoter hypermethylation (O-6-methylguanine–DNA methyltransferase)	- Pyrosequencing - MSP - Array-based methods	- Prognostic	- Recommended for all grade III and IV gliomas	- Anaplastic astrocytoma - Anaplastic oligodendroglia - Anaplastic oligoastrocytoma - GBM - Anaplastic gliomas	- Associated with IDH-1/2 and G-CIMP phenotype - Benefit from TMZ treatment for high-grade gliomas - Prolongs PFS and OS in patients with high-grade diffuse gliomas - Extent of MGMT promotor methylation in glioma WHO grade II depends on IDH mutation and on 1p/19q co-deletion - Important in stratification for clinical trials - Independent favorable prognostic factor in GBM - Sensitivity to alkylating chemotherapy	Staedtke et al. 2016, Kristensen et al. 2019, Jiang et al. 2021, NCCN 2020.

Table I. Continuation.

ATRX loss of function mutations (Alpha-thalassemia/ mental retardation syndrome X)	- IHC - Sanger sequencing - NGS	- Diagnostic	- Strongly recommended for gliomas	- Grade II/III astrocytoma - Secondary GBM	- Frequently present in IDH-mutant astrocytic tumors - Mutations are observed in grade II/III astrocytomas, where they commonly co-occur with IDH mutation and are associated with extremely long telomeres - Whether this is independent of the effects of IDH mutation remains unclear - Relatively favorable prognosis in IDH-wildtype GBM - ATRX variants rarely found with 1p/19q codeletion	Staedtke et al. 2016; Kristensen et al. 2019; Jiang et al. 2021, NCCN 2020.
TERT promoter mutations [Telomerase reverse transcriptase (C228T/C250T)]	- Sanger sequencing - Pyrosequencing - NGS	- Both	- Recommended for gliomas	- Grade II/III astrocytomas - Oligodendrogliomas - GBMs	- Diagnostic parameters for diffuse astrocytic glioma, IDH-wildtype, with molecular features of GBM, WHO grade IV - Present in almost all IDH-mutant, 1p/19q-codeleted oligodendrogliomas - Frequent in IDH-wildtype GBM - Relatively worse prognosis in wild-type IDH or GBMs with unmethylated MGMT - Relatively favorable prognosis in IDH-mutant gliomas	Staedtke et al. 2016, Kristensen et al. 2019, Jiang et al. 2021, NCCN 2020.

Table I. Continuation.

H3 G34 mutation [H3 Histone Family Member 3A (H3F3A)] **H3 K27M mutation [H3 Histone Family Member 3A (H3F3A) or Histone** **Cluster 1 H3 Family Member B/C (HIST1H3B/C)]**	- IHC - Sanger sequencing - NGS	- Diagnositic	- Recommended if clinically appropriate	- Pediatric high-grade gliomas - GBMs in adults	- Occurs most often in high-grade, IDH-wildtype tumors in young patients with glial or embryonal histology - Diagnostic parameter diffuse midline glioma (DMG) H3 K27M-mutant and diffuse glioma H3.3 G34-mutant - Poor prognosis in DMG - Prognostic meaning in other tumors remains unclear - Relatively worse prognosis than that of wildtype diffuse midline gliomas slightly longer survival time than IDH-wildtype GBM, but shorter than IDH-mutant astrocytoma, WHO grade IV - Mutations in the promoter of TERT and ALT secondary to mutations in ATRX are complementary mechanisms for telomere lengthening and are an essential step in gliomagenesis -Potentially predictive of effect of EZH2 inhibitors	Staedtke et al. 2016, Kristensen et al. 2019, Jiang et al. 2021, NCCN 2020.

Table I. Continuation.

EGFR amplification **EGFRvIII** **(Epidermal growth factor receptor)**	- FISH -Digital PCR - Array- or NGS-based methods - RT-PCR - Digital PCR - IHC - MLPA - NGS	- Diagnostic	- Recommended if clinically appropriate	- GBM	- Diagnostic parameters for IDH-wildtype astrocytoma, with molecular features of GBM - High copy number amplification frequent in IDH-wildtype GBM - EGFRvIII present in about half of EGFR-amplified GBMs - Possible therapeutic target	Staedtke et al. 2016, Kristensen et al. 2019, Jiang et al. 2021, NCCN 2020.
BRAF mutation **(BRAF V600E) or fusion (KIAA1549:BRAF)**	- IHC - Sanger sequencing - NGS - Pyrosequencing	- Diagnostic	- Recommended if clinically appropriate	- Pleomorphic xanthoastrocytomas - Gangliogliomas - Pediatric low-grade gliomas - Epithelioid GBMs	V600E mutation associated with: - Diagnostic value for a variety of gliomas, including epithelioid GBMs - Increased response to BRAF inhibitors - Must be interpreted in conjunction with histology and other prognostic factors Fusions associated with: - Indolent tumors - Pilocytic astrocytomas - Possible therapeutic target	Monga et al. 2017, Jiang et al. 2021, NCCN 2020.
Ki67	- IHC - Sanger sequencing - NGS - Pyrosequencing		- Not included in the NCCN recommendations.	- Gliomas	- Conflicting results about glioma prognosis.	Monga et al. 2017, Jiang et al. 2021, NCCN 2020.

*** Recommendations based on NCCN 2020.**

that greater EOR of enhancing tumor tissues can significantly prolong PFS and OS in MGMT promoter methylated GBMs. Whether it can only prolong OS in MGMT promoter unmethylated GBMs or it is regardless of the expression of MGMT promoter warrants further investigation (Li et al. 2020). Methylated MGMT promoter patients had a survival benefit when treated with TMZ and RT, compared with those who received RT only. MGMT promoter-unmethylated patients had no survival benefit from chemotherapy. This is why MGMT promoter methylation testing ought to be made before the clinical decisions being made (Butler et al. 2020, Szopa et al. 2017). Interestingly, approximately 40% of IDH wild-type GBMs and 80% of low-grade diffuse gliomas accompany MGMT promoter methylation (Li et al. 2020). Karschnia et al. (2020) reported that probably due to the IDH mutation to be a more dominant prognostic factor than MGMT promoter methylation, the prognostic value of this last is greater in wild-type IDH astrocytoma patients than those with mutant IDH with or without 1p/19q co-deletion. Collectively, extent of MGMT promoter methylation in glioma WHO grade II depends on IDH mutation and on 1p/19q co-deletion (Karschnia et al. 2020). While in WHO grade III, MGMT methylated is predictive, as patients treated with adjuvant chemoradiation or adjuvant radiation therapy did have improved OS. Though, there was no difference in OS observations amongst patients receiving adjuvant chemotherapy or those patients receiving no adjuvant treatment (Karschnia et al. 2020). Further, Hallaert et al. (2020) observed that the partial resection significantly benefits OS compared to biopsy in wild-type IDH GBM patients with unmethylated MGMT promoter who were treated with surgery followed by chemo-radiotherapy. But more studies are needed to support the information here cited and its application in future management of such patients.

IDH 1/2

The discovery of two key metabolic enzyme mutations, isocitrate dehydrogenase 1/2 (IDH-1/2) happened in 2008, during a genomic analysis of gliomas (astrocytomas and oligodendrogliomas). It was the major breakthrough that broadened understanding regarding glial tumor genesis, biology and diagnosis (Santos et al. 2020). Since then, it is appreciated that IDH-1/2 mutations are associated with a relatively prolonged patient survival for some gliomas and GBM (Molenaar et al. 2018). The wild-type IDH-1 protein is located in the endoplasmic reticulum, peroxisomes and cytoplasm, whereas the IDH-2 is found in the mitochondria (Waitkus et al. 2018). Both enzymes are known for their role in catalyzing the reversible oxidative carboxylation of isocitrate, resulting in CO_2 and alpha-ketoglutarate (αKG) to produce NADPH in the citric acid cycle (Krebs cycle) (Buonaguro et al. 2017, Waitkus et al. 2018). Mutations in genes that encode the IDH-1/2 proteins enable αKG conversion to the oncometabolite 2-hydroxyglutarate (2-HG). Elevated concentrations of 2-HG have been detected in the serum of patients with IDH-mutant gliomas and IDH-mutant acute myeloid leukemia (Waitkus et al. 2018, Buonaguro et al. 2017). Also, the mutations in IDH-1/2 appear to alter the genetic regulation and programming of the tumor cells, causing them to continue to grow and divide and not differentiate into normal cells (Molenaar et al. 2018). Currently, the most common missense mutations, $IDH1^{R132H}$, $IDH\text{-}2^{R140Q}$ or $IDH\text{-}2^{R172K}$ (Waitkus et al. 2018, Buonaguro et al. 2017) can be detected by immunohistochemistry, standard sequencing or genotyping methods (Szopa et al. 2017). The IDH-1/2 mutations are known to occurs early in glioma pathogenesis,

especially in WHO grade II and III astrocytic and oligodendroglial tumors (72–100%), and in secondary GBM (73%–85%), which develop from astrocytoma (Szopa et al. 2017, Kristensen et al. 2019, Fisher & Adamson 2021). These molecular changes have been pivotal in diagnosis and prognosis of patients, as observed in patients with anaplastic oligodendrogliomas IDH-mutant and 1p/19q co-deleted, which typically live 12–14 years; patients with GBM IDH-wild-type, which typically live 12-18 months, while patients with GBM IDH-mutant live 24–36 months; and, patients with anaplastic astrocytoma IDH-1 wild-type, which present similar tumor behavior and prognosis to GBM (Fisher & Adamson 2021). Therefore, concerning the clinical value of IDH mutations, it was verified that these mutations are associated with better OS and PFS, mainly in GBM patients without 1p/19q co-deletion apt for surgical resection (Waitkus et al. 2018, Karschnia et al. 2020). Deng et al. (2018) reported that the frequency of IDH-1/2 mutations differed significantly between gliomas of grades II, II-III, and III, suggesting that such mutations are associated with progression from grade II to III, in which they were more frequent. Thus, it is speculated that patients with grades II-III and IDH-1/2 mutations have a better prognosis than those who do not (Deng et al. 2018). In this context, even if the mutations allow the high amount of 2-HG produced to favor tumor progression, these same mutations increase cellular oxidative stress, reduce NADPH levels and increase the OS of patients, nullifying the negative effect of 2-HG (Kristensen et al. 2019, Karschnia et al. 2020). Interestingly, for astrocytoma patients, it was verified a difference of clinical relevance that Non-IDH1^{R132H} IDH-1/2 mutations are associated with increased DNA methylation and improved survival compared to patients harboring IDH-1^{R132H} mutated tumors (Tesileanu et al. 2021). These data are supported by the fact that increased genome-wide DNA methylation levels are associated with improved outcome in this tumor type and indicate that the type of IDH-1/2 mutation should be taken into account for prognostication of astrocytoma patients (Tesileanu et al. 2021). Due to the growing data regarding the positive association of these mutations and increased OS and PFS, IDH was a molecular marker included in the updated 2016 WHO classification of astroglial brain tumors (Louis et al. 2016). In this context, IDH is the most effective prognostic factor (Butler et al. 2020), especially with radiation or alkylating therapy (Chen et al. 2019) and its ability to predict glioma prognosis exceeds other histological and molecular targets (Butler et al. 2020). But much has to be done yet, further studies and bigger samples are urgently necessary to explain the precise roles of these mutations in brain gliomas.

1p/19q

Another tumor marker included in the updated 2016 WHO classification was the deletion of p-arm of chromosome 1 and the q-arm of chromosome 19 (1p/19q). It is the most common glioma chromosomal change (Butler et al. 2020) and a very frequent mutation in WHO grade II and III oligodendroglial (80–90%), anaplastic oligodendroglioma (50–70%), and anaplastic oligoastrocytoma (20-30%) (Butler et al. 2020). Moreover, it is highly relevant indicator of longer PFS and is an important positive prognostic biomarker (Butler et al. 2020, Yao et al. 2020) in patients undergoing both PCV (procarbazine, lomustine, and vincristine) and TMZ chemotherapy (Chen et al. 2019). This information is relevant because it was observed that chemotherapy in combination with RT present better OS over RT alone in patients with co-deleted oligodendrogliomas (Butler et al. 2020, Yao et al. 2020, Altwairgi et

al. 2017). Furthermore, the diagnostic value of this marker is further enhanced by combined use of other biomarkers such as IDH-1, IDH-2, ATRX, CIC and FUBP1. However, this generally recognized essential diagnostic tool is still not as widely available as desirable. It is due to the fact that it is a comparatively laborious and thus expensive test method (Altwairgi et al. 2017). It is evident that the inclusion of the IDH and 1p/19q status markers contribute to the classification of diffuse gliomas, although they are not sufficient given the heterogeneity of these tumors. Therefore, future editions of the WHO classification of gliomas will be revised and updated to include new molecular markers undergoing studies such as MGMT and/or telomerase reverse transcriptase (TERT) (Davis 2018). For example, in gliomas with wild-type IDH or GBMs with unmethylated MGMT, TERT mutations were found to predict poor prognosis (Davis 2018). Thus, adding more tumor markers to panel investigation, the molecular profile of each tumor will be more detailed and helpful at the moment of therapy decision-making (Sonoda 2020).

ATRX

Numerous studies are carried out to improve the knowledge on the classification and prognosis of glioma also regarding mutations in α thalassemia/mental retardation syndrome X-linked (ATRX) gene and telomerase reverse transcriptase (TERT) (Liu et al. 2019). Concerning ATRX, it was first analyzed to refine the diagnosis of IDH mutant astrocytoma, and it was used to delineate these tumors from oligoastrocytoma and oligodendroglioma (Brandner & Von Deimling 2015). The human ATRX gene alters DNA conformation in order to regulate DNA recombination, repair and transcriptional regulation (He et al. 2017, Liu et al. 2019). When mutations occur in the ATRX gene, certain genetic conditions can be triggered, such as α-thalassemia X-linked mental retardation, mental retardation and thalassemia (He et al. 2017, Liu et al. 2019). Furthermore, osteosarcoma, neuroblastoma, pancreatic neuroendocrine tumors and other types of cancer presented mutations in the ATRX genes (He et al. 2017, Liu et al. 2019). In gliomas, this mutation was firstly found in adolescents and young adults aged 11–30 years. ATRX mutations are present in up to 75-80% of WHO grade II and grade III astrocytomas, where they commonly co-occur with p53 and IDH mutation and are associated with extremely long telomeres (He et al. 2017, Liu et al. 2019). Therefore, mutations in ATRX may not be important only to glioma formation and development, as to possible drivers in gliomagenesis and progression to secondary GBM (He et al. 2017, Liu et al. 2019).

The incorporation of histone H3.3 proteins into the telomeric regions of chromosomes requires the chromatin-remodeling complex ATRX/DAXX (death-associated protein 6) (Olympios et al. 2021). The dysfunction of this complex results in a homologous recombination-mediated mechanism known as alternative lengthening of telomeres (ALT). This suggests that mutations in the promoter of TERT and ALT secondary to mutations in ATRX are complementary mechanisms for telomere lengthening and are an essential step in gliomagenesis (Olympios et al. 2021). Notably, although the prognostic role of mutations in TERT promoter has not been clearly established since there are numerous confusing factors both clinical such as age, initial surgical procedure, and molecular such as IDH mutations, MGMT methylation status, or EGFR amplification (Olympios et al. 2021), this information is a valuable additive tool to assist histological diagnosis in order to refine the 2016 WHO classification, because

the presence of TERT promoter mutation associated with IDH mutation and 1p/19q co-deletion in low-grade gliomas is an indicative of oligodendroglial origin (Siegal 2016, Louis et al. 2016). Interestingly, IDH mutation should be also simultaneously analyzed with ATRX sequencing to reliably classify tumors. However, in order to make it feasible in daily practice, it is suggested the method of immunohistochemical staining to observe ATRX mutations/ALT phenotypes in gliomas (Liu et al. 2019, Olympios et al. 2021). Therefore, tumors with IDH-1/2 mutation with wild-type TERT and loss of ATRX expression can be reliably classified as diffuse astrocytoma, while IDH-1/2 mutant tumors with retained ATRX expression should undergo testing for 1p/19q co-deletion to help differentiate between a diffuse astrocytoma and oligodendroglioma (He et al. 2017, Lipp & McLendon 2018, Liu et al. 2019, Ohba et al. 2020). Even though astrocytoma and oligodendroglioma have the same IDH mutation, it is unknown why the first selects the ALT phenotype and the second, the TERT promoter mutation in order to maintain telomere length and resolve telomeric dysfunction (Ohba et al. 2020).

TERT

Among human tumors, 10-20% utilize alternative pathways for telomere lengthening maintenance, which is a universal hallmark of cancer (Fouad & Aanei 2017). As mentioned previously here, low-grade oligodendroglioma and astrocytoma use TERT expression and the ALT phenotype, respectively (Viswanath et al. 2021). In this sense, TERT promoter mutations are the most frequent cancer genomic alterations. They occur in 51% of all glioma grades, being present in oligodendroglioma (78%), oligoastrocytoma (25%) and astrocytoma (10%) (Powter et al. 2021). Regarding GBM, mutations commonly occur at two hotspots, referred to as C228T and C250T, which are mutually exclusive and occur in 80–90% of GBM patients (Powter et al. 2021). TERT promoter mutations, C228T and C250T, are believed to be associated with genomic instability, telomerase activation, oncogenesis and immortalization of cells (Powter et al. 2021). The gold standard to identify TERT promoter mutations in GBM remains based on molecular characterization of tumor DNA. The identification of TERT promoter mutations traditionally relied on Sanger sequencing, based on tumor DNA sequencing. Alternative sequencing methods were recently developed to increase the mutation detection rate in cases of low mutant allele frequency; these methods include Droplet Digital PCR (ddPCR), mass-spectrometry-based tests, and next-generation sequencing. ddPCR techniques have a higher sensitivity than Sanger sequencing in the detection of IDH1 and TERT promoter mutations (Liu et al. 2019). In addition, as it could not miss, it considered the genotype associating IDH and TERT. Co-IDH- and TERT-mutations are founder mutations of oligodendroglioma genotype, while the genotype of IDH-1/2 mutation with wild-type TERT and loss of ATRX is an astrocytoma genotype. Overall, both IDH and TERT have critical roles in diffuse glioma development, even though TERT seems to contribute differently to the progression of oligodendroglioma and GBM (Lipp & McLendon 2018, Ichimura 2019). Patients with the IDH-TERT promoter double mutations had better OS than those with IDH only mutations (Powter et al. 2021). Indeed, more studies are still necessary to better predict the diagnostic and prognostic role of TERT promoter mutations alone and in association with IDH-1/2, MGTM, ATRX and 1p/19q codeletion. However, the molecular classification of these mutations indicates aggressive behaviors and unfavorable outcomes in GBM, as well as OS and PFS compromised in patients

with glioma (Kim et al. 2018, Liu et al. 2019). Thus, it may better predict patient prognosis and guide clinical treatment strategies in the future.

H3

Another protein that is highlighted for functioning as a tumor marker is the H3 histone family 3A (H3F3A) (Ebrahimi et al. 2019). Mutations in this histone were initially detected in the pediatric (peak incidence between 6 and 8 years) malignant brain stem diffuse intrinsic pontine glioma (80%), with a median survival less than a year (Lowe et al. 2019, Ebrahimi et al. 2019). However, regardless of tumor histology and location, detection of the H3 mutation in midline glial tumors has indicated a poorer prognosis in the pediatric age groups compared to adult patients (Ebrahimi et al. 2019). Diffuse midline glioma, H3 K27M mutant, was recognized as a distinct entity by the 2016 WHO classification (Louis et al. 2016, Graham & Mellinghoff 2020). These tumors comprise approximately half of all pediatric high-grade gliomas. But they also appear in chondrosarcomas and giant cell tumors of bone in adolescents (H3 K36M), and in GBM (5%), in adults (Lowe et al. 2019). The most frequent mutations are the histone H3 K27M with recurrent alterations in PDGFRA and TP53 (Lowe et al. 2019). Overall, the oncohistones H3 K27M (glioma), H3 K36M (chondroblastoma) and H3 G34V/R (both glioma and bone cancers) result in an amino acid substitution at/near a lysine residue, causing impaired DNA methylation that impedes the deposition of histone marks and therefore reprograms the transcriptome that drives gliomagenesis (Siegal 2016, Davis 2018, Larson et al. 2019). Specifically, H3.3 K27M enhances self-renewal of neural stem cells without inducing immortalization, and accelerates hindbrain tumorigenesis, of either medulloblastoma or high-grade glioma from neonatal stem/progenitor cells (Larson et al. 2019). These mutations are also under constant investigation and will be widely useful for future mechanistic and preclinical studies of glioma pathogenesis and therapeutic response.

EGFR

Epidermal growth factor receptor (EGFR) is a transmembrane protein, member of receptor tyrosine kinase family, which plays a crucial role cellular signaling pathways associated with proliferation, survival, metabolism, invasion and metastasis (Saadeh et al. 2018, Hao & Guo 2019). Alterations and mutations of EGFR have been observed in many cancers, such as lung, head and neck, breast and gastrointestinal tract (Hao & Guo 2019). Because of this, EGFR became a potential tumor marker, especially for GBM, where it was found to be overexpressed (60%), amplified (40%) and mutated (24%-67%). Among them, EGFR amplification is a particularly striking feature of primary and secondary GBMs (Hao & Guo 2019, Saadeh et al. 2018), in which it has been described to promote proliferation, invasion, and chemo-radioresistance (Hao & Guo 2019). Also, it is known that EGFR variant type III (EGFRvIII) is the most common mutation of EGFR in GBM (Saadeh et al. 2018). In this sense, Guillaudeau et al. (2012) associated both, EGFR amplification and high levels of EGFRvII, vIII and vIV (different products of gene splicing) in GBM. While other studies reported EGFR amplification to be present also in anaplastic oligodendrogliomas (AOs), anaplastic oligoastrocytomas and EGFRvIII in GBM and AO (Saadeh et al. 2018). In this respect, EGFRvIII overexpression in the presence of EGFR amplification is an independent and the strongest poor prognostic factor for OS, playing a pivotal role in enhanced gliomagenesis (Hao & Guo 2019). However, more studies are necessary, since a conclusive consensus on EGFR gene

overexpression and its varied mutations as molecular markers of prognosis could not be reached yet. In low-grade gliomas, although EGFR amplification is rare, it was correlated to the higher malignancy grade and lower OS (Saadeh et al. 2018), at the same time that EGFR mutation indicates increasing infiltration of specific types of immune cells and poor prognosis (Hao & Guo 2019). Concerning EGFR and its relation with other tumor markers, EGFR amplification and MGMT promoter methylation were associated with better response and OS after RT alone rather in IDH-wild-type GBM patients than those without EGFR amplification. Nevertheless, the facts that explain why it did not occur after chemo-radiotherapy are not fully clarified. In contrast, TERT mutations had no impact on prognosis (Brito et al. 2019). Concerning EGFR and its relation with others tumor markers, EGFR amplification in GBM IDH-wild-type patients was associate with a better RT response and better OS than those mutated. Additionally, EGFR expression evaluation was shown to refine the prognostic value of MGMT methylation status in GBM (Kim et al. 2021). Altogether, current data regarding EGFR mutation and amplification ought to be validated with bigger cohorts and additional studies to determine whether EGFR protein expression is a better biomarker than EGFR amplification for clinical decisions and trial enrollment (Brito et al. 2019).

BRAF

V-raf murine sarcoma viral oncogene homolog B1 (BRAF) gene encodes the protein B-raf, a key member of the RAF/MEK/ERK signaling axis of the MAPK pathway, which is related to cellular proliferation and survival (Kowalewski et al. 2020). Mutations in MAPK pathways components, specially BRAF gene, have been described in melanoma, colorectal, thyroid and non-small-cell lung cancers and hairy cell leukemia (Kowalewski et al. 2020). Regarding to CNS cancers, mutations in BRAF have been shown to contribute to tumor development and progression, being rare in adult gliomas and more predominant in pediatric gliomas (Da et al. 2021). It accounts for 80% of pleomorphic xanthoastrocytomas, 20%-60% of gangliogliomas, 85% of pediatric low-grade gliomas, 5%–15% of adult low-grade gliomas, 20% of pediatric GBMs, 3% of adult GBM and 50% of epithelioid GBMs, being the most known of them, the point mutation BRAF V600E (Monga et al. 2017, Behling & Schittenhelm 2019, Da et al. 2021). In BRAF V600E variant, there is the substitution of thymine by adenine at nucleotide 1799, resulting in the substitution of valine for glutamic acid at amino acid 600 and subsequent BRAF kinase overaction and ERK signaling hyperactivation. BRAF amplification can also cause MAPK signaling hyperactivation, what harms therapies seeking to inhibit this signaling in BRAF V600E tumors (Kowalewski et al. 2020, Da et al. 2021). As tumor molecular marker, the BRAF mutation has a role in the diagnosis and monitoring of the treatment of gliomas. For example, its detection indicates a slow progress of pilocytic astrocytoma; the variant BRAF V600E can be found in both low and high-grade gliomas; it was observed increased survival in pediatric patients and younger adults (<35 y) harboring this variant; and tumors with BRAF V600E have indication of targeted therapy, with a BRAF inhibitor in clinical trials (Aquilanti et al. 2018, NCCN 2021). Other BRAF mutation, the tandem duplication at 7q34 creates the fusion gene KIAA1549:BRAF, which also impairs the correct activation of MAPK signaling, leading to dysregulated cell growth, differentiation and apoptosis. The prognostic significance of both BRAF mutation/amplification, as to all biomarkers cited here, require more studies to clearly describe their role in glioma patients

as well its relation with other biomarkers findings (Staedtke et al. 2016, Da et al. 2021). Due to mentioned aspects, BRAF become an attractive therapeutic target, although there are any promising results in relation to CNS tumors yet (Staedtke et al. 2016). Due to the need for further studies, the evidence of benefit from BRAF-targeted therapy as a first-line treatment in CNS tumors is not strong and is still under investigation (Kowalewski et al. 2020).

Ki67

The Ki67 DNA-binding nuclear protein, detectable during active phases of the cell cycle, is the most used glioma proliferation molecular marker (Nielsen et al. 2018), whose index correlates with the clinical course of several types of cancer and the histological grade of grade II–III or grade IV gliomas (Hu et al. 2013). Specifically, it has been shown that an increased level of Ki67 proliferation index is associated with increased risk of tumor recurrence and dimension (Armocida et al. 2020). In this context, Ki67 expression is a predictive factor for poor prognosis of glioma grade II-III, but more studies are urgent to better clarify its role in GBM. Many studies have attempt to relate Ki67 and patients OS as well as its prognostic value, but tumor heterogeneity has been a major limitation, in addition to the possible variable expression of Ki67 protein from area to area of analyzed tumor and many other factors of analytical methods, clinical elements of uncertainty and conflicting results (Armocida et al. 2020). Contrary, recent studies with GBM patients showed the correlation of high Ki67 index and longer OS of these patients and that it might be due to the susceptibility of these patients to adjuvant therapy (Alkhaibary et al. 2019). Generally, more studies, with a larger number of patients and longer follow-ups are necessary to stablish a reliable threshold for Ki67 outcomes and better guide these laboratory analyses.

GLIOMA MOLECULAR MARKERS RECOMMENDED BY WHO

According to updated WHO 2016 classification of tumors of the CNS, some already known and other new molecular markers have become clinically significant for glioma diagnostic and prognostic (Louis et al. 2016). Therefore, to keep up with advances in the area of molecular biology, it became valid to update the classification and guidelines for the management of CNS tumors (Louis et al. 2016). Thus, a Consortium to Inform Molecular was established and Practical Approaches to CNS Tumor Taxonomy to ease the process of organizing new relevant information about CNS tumors and how such information should be implemented in clinical practice (Louis et al. 2016). There are no glioma subtypes which are discouraged to be tested through molecular markers by guidelines, neither biomarkers testing discouraged, since patients with a mutation panel can be treated with more targeted therapy and that such a panel supports their classification, improving the accuracy of their diagnosis and prognosis stratification (NCCN 2020, Louis et al. 2021). The same recommendations are suggested in Brazilian legislation and are present in the Diagnostic and Therapeutic Guidelines for Brain Tumor in Adults of Brazilian Health Minister (Brazil 2020b). However, in Brazil, glioma molecular markers are still not a *sine qua non* condition for diagnosis and treatment (Brazil 2020b). Therefore, till 2020, the National Comprehensive Cancer Network (NCCN) Guidelines recommendations for WHO grade I, II, III, and IV gliomas strongly recommended approaches include: (i) IDH, ATRX, TERT, 1p/19q testing; (ii) immunohistochemistry for most common IDH-1 ^{R132H} mutation; (iii)

sequencing for less common IDH-1/2 mutations in proper clinical context; (iv) tumors without an IDH mutation should not be regarded as 1p/19q co-deleted, even when results suggest otherwise; and (v) MGMT promoter methylation status for grades III-IV gliomas. Lastly, the recommended approaches include: (i) 1p/19q testing is not necessary in tumor that are definitely IDH wild-type; (ii) H3 mutations and BRAF fusion/mutation may be carried out as clinically indicated.

GLIOMA THERAPY AND TUMOR MARKERS EXAMS THROUGH SUS ASSISTANCE IN BRAZIL

SUS is the world largest universal healthcare system, funded by federal, state, and municipal resources and serves over SUS serves approximately 150 million people (80% of the Brazilian population), consuming 45% of the country's total health expenditure (IBGE 2019). While the supplementary health sector, represented by health plans, has 40 million users (20% of the Brazilian population), consuming 55% of this total expenditure (IBGE 2019). These data clearly reinforce the need for better financing of the public system, as well as qualifying the service network for its users and, at the same time, guaranteeing the reduction of inequity in access. Thus, in order to masterfully use health planning and assessment instruments, in 2011, SUS managers implemented the Access and Quality Improvement Program (PMAQ) (Brazil 2012b). From this, the work aimed at the decentralization of the SUS has provided more adequate models and assessment systems for regional health problems and identified the health determinants involved, thus supporting a management of health services increasingly focused on needs. of the population. Even though, the situation of SUS financing in recent years and changes in structuring policies create uncertainties about the future of health in the country (Vieira 2020). Within this context, literature is absent about how SUS started covering tumor marker first exams. Currently, since the simplest exams (eg, blood count) are offered, including even highly complex ones (eg, tumor markers). The amounts earmarked, both for the coverage of primary health care, as well as for medium and high complexity procedures, are present in the pluriannual and annual budget plans of SUS (Vieira 2020). Public spending *per capita* on health increased between 2010 and 2018. However, compared to 2014, it reduced 3% in 2018. There was a displacement of the allocation of federal resources to the detriment of transfers to the states (-21%) (Vieira 2020). With regard to primary care, parliamentary amendments generated changes in the policy and expansion of allocated resources, which culminated in an increase in spending on public resources (Vieira 2020). In relation to pharmaceuticals, this increase was due to the centralization, in Brazilian Ministry of Health, of the purchase of items with high budgetary impact, such as new medications, vaccines, blood products and judicialization (Vieira 2020).

In Brazil, it would be advantageous for the SUS to seek to make available the search for tumor markers in its table of procedures since, after further future studies, they can also be classified as important in the early diagnosis of neoplastic conditions, and the best supportive care can be the course more suitable for some patients (Jiang et al. 2021). This would lead to the early discovery of cancer, allowing treatment to be started in its early stages, with less invasive and aggressive treatments, as well as an increase in the effectiveness of the therapy and in the survival of the patient. The evaluation of tumor markers could generate a national database, generating scientific studies with a larger

number of patients that would allow greater knowledge of new specific target therapies as well as information on carcinogenesis and predictive and prognostic biomarkers.

SUS is organized based on regions and networks (Santos 2017). It is known that the financial transfer must be carefully planned and also updated periodically to serve all the offices it covers. This need is justified, for example, by the fact that hospitals, laboratories and doctors have a table that contains all the tests that SUS finances (Albuquerque & Viana 2015). In view of the objective of clarifying the bureaucratic obstacles in the request for exams of tumor markers by SUS, this important step from screening and diagnostic investigation to therapeutic monitoring and recurrence of the various types of cancer, in the flowchart below (Figure 2), it is verified that in the Basic Health Unit (UBS), patients are screened. That is, in the face of the suspicion of the patient, this is the first place he/she should go (Oncoguia 2015).

When cancer is suspected at the UBS, the patient is referred to a specialist doctor at the Center for Medical Specialties (CES), who is responsible for requesting tests from the accredited laboratories, after undergoing an audit following the Manual of Technical Bases of Oncology (Brazil 2019). In any case, other specific tests must also be evaluated, since the

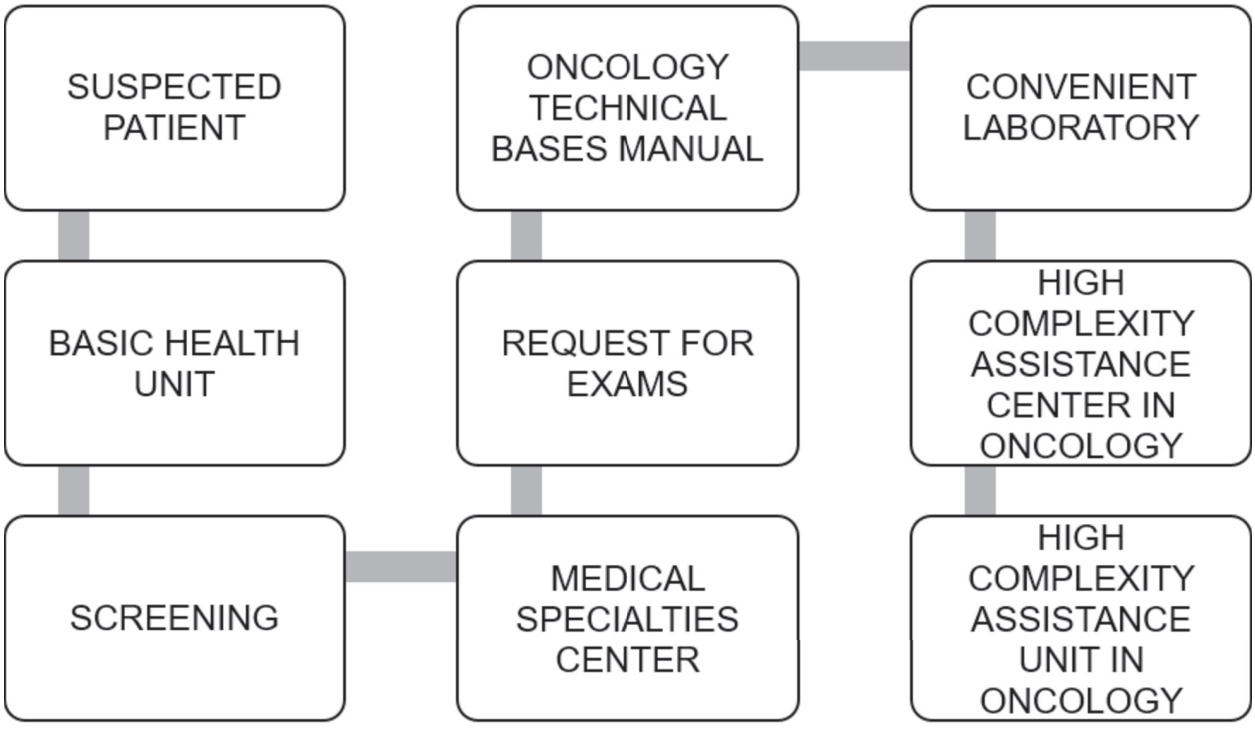

Figure 2. Steps for requesting the examination of tumor markers at SUS. It starts at the UBS, where a screening of patients is carried out. Once the suspicion of cancer is found, at the UBS, the patient is referred to a specialist physician at the CES, who is responsible for making the request for examinations to the partner laboratories, after undergoing an audit following the Oncology Technical Basics Manual. Anyway, other specific tests must also be evaluated, since tumor markers can also be altered in physiological conditions other than cancer. Afterwards, the CES specialist physician assesses the laboratory reports and refers the patient to services linked to the SUS and which carry out cancer treatment. The CACONs and UNACONs must be registered with the Ministry of Health and are coordinated by the INCA. The CACON and UNACON may be part of a public or philanthropic hospital, in charge of diagnostic confirmation, staging, outpatient and hospital care, oncological emergencies and palliative care.

tumor markers may also be altered in other physiological conditions than cancer (Sokoll & Chan 2020). In sequence, the CES specialist physician evaluates the laboratory reports and sends the patient to the services linked to SUS and which carry out cancer treatment (Oncoguia 2015). The CACONs and UNACONs must be registered with the Ministry of Health and are coordinated by the INCA (Oncoguia 2015). The CACON and UNACON can be part of a public or philanthropic hospital, responsible for "[...] diagnostic confirmation, staging, outpatient and hospital assistance, care for oncological emergencies and palliative care" (Oncoguia 2015). It cannot be ignored that, since it is a laboratory procedure, strict quality control must be present in all stages of the process. Overall, SUS provides the Diagnostic and Therapeutic Guidelines (DDT) for the treatment of Cerebral Tumor in Adults, at the Ministry of Health, through Ordinance No. 599, Of June 26, 2012 (Brazil 2020b), which guides the best procedures in the area of Oncology. Here it is important to mention that, due to the differentiated system of financing of procedures and treatments in oncology, this document is not restricted to the technologies incorporated in SUS, but to what can be offered to this patient, considering the financing transferred to the centers of attention and their autonomy in choosing the best option for each clinical situation (Brazil 2018).

It is important to clarify that cancer care in SUS does not constitute pharmaceutical assistance, which in general and mistakenly, is usually summarized in cancer treatment. In other words, if a patient seeks a clinical analysis laboratory to perform exams for tumor markers, used for the best therapeutic maintenance, the laboratory registered with SUS, if it does not perform the requested exam, should seek another partner laboratory in the SUS network that does it (Brazil 2019).

The oncology assistance provided by SUS is not included in the Pharmaceutical Assistance block, but in the Medium and High Complexity Health Care (MAC) block and is reimbursed through specific procedures (surgical, RT, chemotherapy and iodotherapy) (Brazil 2018). Therefore, these procedures are reported as chemotherapeutic procedures in the APAC subsystem (authorization of highly complex procedures), of the SUS Outpatient Information System (SIA-SUS), and must be provided by the health institution accredited by SUS and qualified in Oncology, being reimbursed according to the APAC code (Brazil 2018). It is important to inform that for the patient to have access to cancer treatment by SUS, he must be enrolled in a health facility authorized by SUS in the area of High Complexity in Oncology, in the region where he lives and be accompanied by the medical team, who will prescribe the treatment according to previously standardized clinical protocols (Brazil 2018).

In order to speed up the treatment of cancer in Brazil, in November 2012, Law 12.732 (Brazil 2012a) was sanctioned, then regulated by ordinance 874 of May 16, 2013 (Brazil 2013). This law establishes the maximum period of sixty days for the patient to start treatment at SUS (Brazil 2013). However, in 2019, 39.9% of cancer cases still start after more than 60 days (Table II) (Brazil 2020a).

In general, cancer treatment in Brazil is governed by the National Policy for the Prevention and Control of Cancer, and brain tumors in adults are governed by Joint Ordinance No. 7, of April 13, 2020, which regulates the comprehensive care provided to the patient. by SUS, in a decentralized and regionalized manner in the aforementioned UNACON and CACON. Importantly, these are institutions where these tests are requested, thus establishing the relationship between SUS and molecular

Table II. Distribution of central nervous system cancer cases according to year of diagnosis and time to first treatment. Brazil, 2013 to 2019.

Year	Time to first treatment (days)				Total
	0 to 30	31 a 60	> 60	No information	
2013	37,4 (1036)	20,0 (554)	42,6 (1178)	0 (0)	100,0 (2768)
2014	34,0 (924)	22,0 (597)	44,0 (1196)	0 (0)	100,0 (2717)
2015	32,7 (892)	22,0 (600)	45,4 (1239)	0 (0)	100,0 (2731)
2016	30,6 (897)	23,8 (699)	45,5 (1335)	0 (0)	100,0 (2931)
2017	28,7 (827)	22,0 (663)	48,3 (1394)	0 (0)	100,0 (2884)
2018	31,1 (1567)	14,7 (741)	26,8 (1349)	27,5 (1384)	100,0 (5041)
2019	33,8 (1468)	11,6 (504)	14,6 (634)	39,9 (1733)	100,0 (4339)

***Data available until 2019. Source: adapted from Brazil 2020a. %(N). N = Number of patients.**

markers of gliomas (Brazil 2018). Therefore, the importance of constantly updating the parameters addressed in the Brazilian guidelines and legislation regarding diagnosis, treatment and follow-up of patients with brain tumors is highlighted, in order to ensure their safety, the effectiveness and reproducibility of scientific methods and the quality-of-care conduct and protocols (Brazil 2018, 2020b). Remarkably, in the Brazilian legislation in force in 2020, only the 1p/19q co-deletion markers, mutations in the IDH-1/2 gene and MGMT methylation are cited as the main molecular genetic markers related to the diagnosis of gliomas, based on the Classification of WHO 2016 and scientific bases of studies that reported measures of diagnostic accuracy or those in which it was possible to calculate them, not being discussed about other markers for gliomas (Brazil 2020b).

CLINICAL TRIALS AND FUTURE PERSPECTIVES ON MOLECULAR MARKERS-BASED THERAPIES

The advances regarding molecular markers-based therapies have been made in the sense of understanding the tumor characteristics for future clinical trials designing more than the discovery of new promising drugs. Promisingly, immunotherapy for the treatment of GBM with DCVax-L, which is in phase I/II trials testing and whose patients have presented increased median life expectancy. In this context, further immunotherapy guided by biomarker profile shed some light to improve the management of GBM patients (Szopa et al. 2017). Advances have also been made on the utility of molecular biomarkers in guiding the stratifying of patients for clinical trials eligibility. But the main challenge here is to obtain the most adequate tissue for proper analyses, mainly due to difficulties with tumor localization access. For this reason, it is necessary first standardize the methods of biopsy and biomarkers testing, before to therapeutic innervations testing. Another challenge is the difficulty in obtaining larger groups of patients for clinical trials and maintaining longer follow ups for more accurate results (Monga et al. 2017). What the scientific community has so far most concretely in relation to biomarkers guiding the therapy of patients with glioma, are the present on recommendations of 2016

update of the WHO Classification of Tumors of the Central Nervous System and the subsequent recommendations of the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy — Not Officially WHO. According to these guidelines, briefly, surgery is the primary modality of treatment modality for diffuse gliomas. After that, it is recommended the "watch-and-wait" strategy. If further therapy is necessary, it is used the SOC protocol as previously discussed in this work, taking into consideration the molecular markers profile individually, as well the treatment at recurrence depending on neurological status, patterns of progression and the initial treatment. The chemotherapy as frontline therapy might be an option if neither RT nor maximal resection are feasible (Weller et al. 2021).

CONCLUSIONS

Cancer, especially which affects the brain of the individual, is a complex chronic condition, of an epidemic character, with high rates of morbidity and mortality. It affects and modifies the health of the patient, his family and community, regardless of whether this individual is in a country considered to be developed or not. Its diagnosis, treatment and prognosis depend on the detection of bioindicators and their interrelation so that patients have access to adequate health services. In this sense, to achieve adequate management against the cancer public health problem, a facilitating strategy for the diagnosis, therapeutic control and prognosis of gliomas was included in the Brazilian health system. The tests that detect the biomarkers IDH, 1p/19q, MGMT, ATRX, TERT, H3, EGFR, BRAF and Ki67 are fundamental instruments to manage and direct cancer patients and promote greater and more adequate access for these users in the SUS care sectors. However, for access to these tools to be universal, comprehensive and equitable, there are still many challenges for this patient. For example, there is a need for more studies that correctly predict the applicability of each marker, as well as financial resources from the federal government that enable the availability of the latest technologies to detect these in the provision of public health services. Therefore, this is possible through the planning and organization of the management of public health systems, clinical studies to detect tumor markers in larger and more diverse populations and constant updating of reference units for cancer services, with minimal human and technological resources to carry out diagnostic and treatment tests.

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