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Molecular Biomarker Testing for the Diagnosis of Diffuse Gliomas

Guideline From the College of American Pathologists in Collaboration With the American Association of Neuropathologists, Association for Molecular Pathology, and Society for Neuro-Oncology

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METHODS USED TO PRODUCE THE GUIDELINE

Panel Composition

The College of American Pathologists (CAP) in collaboration with the American Association of Neuropathologists (AANP), Association for Molecular Pathology (AMP), and Society for Neuro-Oncology (SNO) convened an expert panel (EP) consisting of 13 pathologists, two oncologists, one patient advocate, and a research methodologist consultant to develop the guideline. Of the 17 expert panel members, six represented the CAP, two represented ASCO, two represented SNO, two represented AANP, and three represented AMP. The CAP approved the appointment of the project chair and panel members. The EP members performed the systematic evidence review, drafted the recommendations, evaluated the public comments, revised the recommendations, and contributed to the manuscripts.

An advisory panel (AP) of seven pathologists, one oncologist, one molecular technologist, one clinical cytogeneticist, and a patient advocate also helped in the development of the guideline. The role of the AP members was to provide guidance and feedback on the scope and key questions for the literature search, vet the draft guideline statements prior to the public comment period, and to review and provide feedback for the manuscript and supplemental digital content (SDC).

The collaborating societies identified representatives for the expert panel, informed relevant stakeholders within their societies about the open comment period and encouraged them to participate, and approved the manuscript and supplement prior to publication.

Conflict of Interest (COI) Policy

Prior to acceptance on the expert or advisory panel, potential members completed the collaborative COI disclosure process, whose policy and form (in effect June 2017) require disclosure of material financial interest in, or potential for benefit of significant value from, the guideline's development or its recommendations 24 months prior through 12 months post-publication. The potential members completed the COI disclosure form, listing any relationship that could be interpreted as constituting an actual, potential, or apparent conflict. Each potential expert panel member's disclosures were assessed by a COI review committee and categorized as:

No Relevant Conflicts of Interest: Individuals with no relevant COI are approved for full participation including determining the scope and questions to be addressed, reviewing and discussing the evidence, formulating and grading recommendations, voting on recommendations, and writing the document. Research funding that is free of direct or indirect industry funding or control, such as that provided by a government program or a non-profit organization that does not receive industry funding and uses an award mechanism and oversight that is independent of industry, is not regarded to be a conflict of interest. Service on a data and safety monitoring board for such research is also not regarded as a conflict of interest. Finally, industry funded research unrelated to the content of the *Recommendations* is not regarded as a conflict of interest.

Manageable Conflicts of Interest: Individuals with manageable conflicts must disclose their conflicts to the whole guideline panel (done via report at every meeting). They may participate in discussions about the evidence, but must excuse themselves or be recused from decision-making, including formulating, voting on, writing, and grading recommendations related to their COI (i.e., recommendations addressing a product of the commercial entity with which they have a relationship or addressing a product of a competitor of the commercial entity with which they have a relationship). COI that require management include:

    A.   Research funding from an industry grant that is paid to the participant's institution and related to the content of the *Recommendations*;

B. Research funding from a government program or non-profit organization that receives funding from industry with business interests in the content of the *Recommendations*;

C. Participation on a data and safety monitoring board concerned with research that is relevant to the content of the *Recommendations* and is funded by an industry with business interests in the content of the *Recommendations*, or by a government program or non-profit organization that receives funding from industry with business interests in the content of the *Recommendations*.

D. Participation in scientific advisory board or consultant activities that are exclusively scientific in nature (i.e., does not involve any activities that could be perceived as promotional) related to the subject matter of the *Recommendations.*

E. Participation in industry-funded research, scientific advisory committees, consulting roles, non-promotional speaking engagements, or expert testimony on matters that are unrelated to the subject matter of the *Recommendations*, but the company involved is known to have business interest in the subject matter;

F. Delivery of non-promotional talks in which the speaker has full control of the content and is either unpaid or paid by a third party that is responsible for ensuring that the event is free of influence of relevant industry (i.e. if the event has industry financial support, all planning and content must be free of industry influence, and any payment of expenses and honoraria must occur through a third party, such as the medical society or institution sponsoring the event, or an event manager acceptable to them, rather than directly by a commercial entity with an interest in guideline subject matter or its agent);

G. Professional roles or activities (i.e., roles and activities performed as part of an individual's profession, whether reimbursed or not) that place an individual in a position to personally gain or lose depending upon the recommendations.

Disqualifying Conflicts of Interest: Disqualifying conflicts of interest include the following:

A. A direct financial relationship with a relevant commercial entity that has an interest in the content of the *Recommendations*, exclusive of the research, data safety monitoring board activities, and scientific advisory board and consultant activities noted above. Such direct financial relationships include the following, whether paid to or held by the individual directly or issued to another entity at the direction of the individual (such as to a panelist's institution):

 i. Payment of wages, consulting fees, honoraria, or other payments (in cash, in stock or stock options, or in kind) by a relevant company as compensation for the individual's services or expertise, exclusive of the research and data safety monitoring board activities noted above. Examples of such services are: participation on scientific advisory committees or consulting that is, in full or in part, promotional in nature; non-continuing medical education (CME) speaking engagements and inclusion in speaker bureaus where control of material is held by industry; expert testimony on matters related to guideline content provided on behalf of a relevant company or a law firm representing a relevant company; employment by a relevant commercial entity (such as a relevant pharmaceutical or medical device company or a third party payer exclusive of commercial laboratory employment that has financial interests in the content of the *Recommendations*).

 ii. Investments in relevant companies by the panelist or the panelist's spouse or life partner (exclusive of general mutual funds).

B. A patent or other intellectual property that is relevant to the *Recommendations'* subject matter and has resulted or could result in payments to the panelist or the panelist's institution.

All panel members were required to disclose conflicts prior to beginning and continuously throughout the project's timeline.

Funding
The CAP provided funding for the administration of the project; no industry funds were used in the development of the guideline.

Disclosures of interest judged by the oversight group as manageable conflicts are listed in the manuscript. Appendix 1 in the manuscript also includes a table of all disclosed interest of the expert panel members during the development of the guideline for complete transparency.

Systematic Evidence Review (SER)
The objective of the SER was to identify articles of sufficient quality that would provide data to inform the recommendations. The scope of the SER and the key questions (KQs) were established by the EP and AP in consultation with the methodologist prior to beginning the literature search. Inclusion and exclusion criteria were determined a priori, and these criteria were applied during each phase of the systematic review.

Search and Selection
A comprehensive literature search was performed in Ovid Medline and Embase.com on 11/13/2017. The search was limited to 1/1/2008 – 11/13/2017. The database searches used standardized database terms and keywords for the concepts of diffuse gliomas, biomarkers or gene alterations, and laboratory test methods. Search results were limited to English language, and the Cochrane search filter for humans was applied.[1] A publication filter to exclude letters, commentaries, editorials, case reports, and conference abstracts was added. Results of both searches were combined, and duplicate references were removed. A literature search refresh was completed in Ovid Medline and Embase.com on 9/3/2019. The search strategies for both databases can be found in Supplemental Figure 1.

A search for grey (unindexed) literature supplemented the initial database searches and included a review of ClinicalTrials.gov, Cochrane Library, Guidelines International Network, National Guideline Clearinghouse, Trip search engine, University of York Centre for Reviews and Dissemination-PROSPERO, and applicable U.S. and international organizational websites. Expert panel recommendations were investigated and added only if they aligned with pre-defined inclusion/exclusion criteria.

A targeted search for pediatric glioma, infantile-type hemisphere glioma, and diffuse pediatric-type high grade glioma and relevant mutations or amplifications was performed in Ovid Medline on July 24, 2020, to ensure relevant evidence was captured for these entities in anticipation of the World Health Organization (WHO) 2021 update. The search was limited to publication dates 1/1/2016 – 7/24/2020, English language, and human studies. Case reports, commentaries, editorials, and letters were excluded.

PRISMA diagrams are included as Supplemental Figures 2 and 3 to depict the outcome of the systematic literature review and the targeted search review.

Outcomes of Interest
The clinical outcomes of interest included survival rates (overall, 1- and 3- year survival, progression free), recurrence rates, response to treatment, and accuracy of diagnosis. The pathologic outcomes of interest included sensitivity, specificity, positive predictive value, negative predictive value, concordance, turnaround time, reproducibility of the various tests, and mutation/alteration/deletion status (percent, presence, frequency, and association with other

alterations). Of the included outcomes, overall survival, accuracy of diagnosis, sensitivity and specificity of testing, and reproducibility of testing were all rated as critical for decision making by the EP. The other outcomes were rated as important.

The following genetic and molecular alterations were of interest:
1. *Isocitrate Dehydrogenase (NADP(+))1 (IDH1)* and *Isocitrate Dehydrogenase (NADP(+))2 (IDH2)* mutations
2. Histone H3 gene mutations
3. *B-Raf proto-oncogene (BRAF)* alterations
4. *ATRX chromatin remodeler (ATRX)* alterations
5. *Tumor protein p53* (*TP53*) alterations
6. 1p/19q co-deletion
7. Chromosome 7 gain
8. Chromosome 10 loss
9. *MYB proto-oncogene* (*MYB*) and *MYB-like* (*MYBL1*) alterations
10. *Telomerase Reverse Transcriptase (TERT)* promoter mutations
11. *Fibroblast Growth Factor Receptor (FGFR)* alterations
12. *Epidermal Growth Factor (EGFR)* alterations
13. *Platelet Derived Growth Factor Receptor Alpha PDGFR Alpha (PDGFRA)* alterations
14. *C-MET (C-MET)* alterations
15. *Cyclin Dependent Kinase Inhibitor 2A (CDKN2A)* alterations
16. *O-6-Methylguanine-Deoxiribose Nucleic Acid Methlytransferase (MGMT)* promoter alterations
17. *Phosphatase and Tensin Homolog (PTEN)* alterations
18. *Neurofibromin 1 (NF1)* alterations
19. Microsatellite instability (MSI) status
20. *MDM2 Proto Oncogene (MDM2)* alterations
21. *Cyclin Dependent Kinase 4 (CDK4)* alteration

Data Extraction & Management
The data elements from an included article/document were extracted by one reviewer into standard data formats and tables developed using the systematic review database software, DistillerSR (Evidence Partners Inc., Ottawa, Canada); a second reviewer confirmed accuracy and completeness. For data extraction, the methodology consultant was the primary extractor, and a second reviewer (an EP member) audited the work. EP changes during the audit were deemed "final." A bibliographic database was established in EndNote (Thomson Reuters, Carlsbad, CA) to track all literature identified and reviewed during the study. Complete extracted data from all studies can be found on the CAP website www.cap.org.

Literature Review and Analysis
The EP met 16 times through teleconference webinars from July 7, 2017, through March 25, 2020. Additional work was completed via electronic mail. The panel met in person September 9, 2017 to confirm the project scope and key question and September 7 and 8, 2018 to review evidence from the systematic review and draft recommendations.

The EP sought to answer what ancillary tests are needed to classify diffuse gliomas and sufficiently inform the clinical management of patients.

All EP members participated in the systematic evidence review (SER): title-abstract screening, full-text review, and data extraction. A dual review was performed for each study and in each phase of the SER; the chair adjudicated all conflicts. A literature refresh was also conducted, where studies also underwent dual review. A total of 86 studies comprised the final body of studies included in the SER. Supplemental Figure 2 displays the results of the literature review. Although data was extracted from 188 studies, many studies reported on alterations in DG

subtypes no longer recognized by the WHO and without sufficient raw data to translate the results into the newer WHO entities. These studies were considered by the EP when drafting recommendation statement but were ultimately used as background and not to inform the statement themselves. All articles were available as discussion or background references. All members of the EP participated in developing draft recommendations, reviewing open comment feedback, finalizing and approving the final recommendations, and writing/editing of the manuscript.

Peer Review

A public, open access comment period was initially held from September 9-30, 2019 on the CAP Web site www.cap.org for any interested stakeholder to provide feedback on the draft recommendations. To increase the number of participants, the comment period re-opened from October 11-31, 2019. Thirteen draft recommendations, two demographic questions, and one question pertaining to the guideline visual aid were posted for feedback. An announcement was sent to the following societies deemed stakeholders:

Medical Societies and Healthcare Organizations/Programs

- American Association of Neuropathologists (AANP)
- American Society for Clinical Pathology (ASCP)
- American Society of Clinical Oncology (ASCO),
- Association of Community Cancer Centers (ACCC)
- Association of Director of Anatomic and Surgical Pathology (ADASP)
- Association for Molecular Pathology (AMP)
- Association of Pathology Chairs (APC)
- Canadian Association of Pathologists (CAP-APC)
- Canadian IHC Quality Control (CIQC) Program
- Canadian Partnership Against Cancer
- Children's Oncology Group (COG)
- College of American Pathologists (CAP)
- Deutsche Gesellschaft fur Neuropathologie und Neuroanatomie (DGNN.DE)
- Eastern Cooperative Oncology Group (ECOG)
- European Society for Medical Oncology (ESMO)
- European Society of Pathology (ESP)
- International Academy of Pathology (IAP)
- International Society for Immunohistochemistry and Molecular Morphology (ISIMM)
- Kaiser Permanente
- National Academy of Medicine
- National Comprehensive Cancer Network (NCCN)
- Nordic Immunohistochemical Quality Control (NordicQC) Program
- Royal College of Pathologists
- Society for Neuro-Oncology (SNO)
- Society of Surgical Oncology (SSO)
- Society to Improve Diagnoses in Medicine (SIDM)
- Southwest Oncology Group (SWOG)
- United States & Canadian Academy of Pathology (USCAP)
- World Health Organization (WHO)

Government

- Centers for Medicare & Medicaid Services (CMS)
- Centers for Disease Control and Prevention (CDC), Division of Laboratory Systems (DLS)
- CDC, Laboratory Medicine Best Practices (LMBP)

- National Medical Products Administration (NMPA)European Medicines Agency
- National Institute for Health and Care Excellence (NICE) (UK)
- NIH, Center for Strategic Scientific Initiatives (CSSI)
- NIH, Division of Cancer Treatment and Diagnosis (DCTD)
- US Food and Drug Administration (FDA)
- Veteran's Affairs (VA)
- Department of Defense (DOD)

Patient Advocacy Groups
- The American Brain Tumor Association
- Dragon Master Foundation
- National Brain Tumor Society
- Prevent Cancer Foundation
- Cancer Leadership Council
- Union for International Cancer Control
- American Childhood Cancer Organization
- The National Children's Cancer Society
- Starlight Children's Foundation

One hundred fifty-five individuals participated in the comment period. "Agree," Agree with modification," "Disagree," and "Neutral" responses were captured for every proposed recommendation and good practice statement. Three hundred and fifteen written responses were also collected. Two recommendations received greater than 90% agree/agree with modification. Six recommendations received 80-90% agree/agree with modifications. Five recommendations received 70-80% agree/agree with modification. The EP members read all the comments and discussed as a group to determine if any recommendations needed revisions.

Decisions were obtained by majority consensus of the panel using nominal group technique (discussion at an in-person meeting, rounds of teleconference webinars, email discussion, and multiple edited recommendations) amongst the panel members. The final recommendations were agreed upon by the EP with a formal vote. The panel considered laboratory efficiency and feasibility throughout the entire considered judgment process. Of those responding to question during the comment period, 38.89% (35 of 90) responded that the entire guideline was feasible, 58.89% (53 of 90) responded that parts of it were feasible, and 2.22% (2 of 90) responded that none of it was feasible. Neither formal cost analysis nor cost effectiveness models were performed.

An independent review panel was assembled to review and approve the guideline on behalf of the CAP Council on Scientific Affairs. The independent review panel was masked to the EP and to each other and were vetted through the COI process.

Quality Assessment Methods
A risk of bias assessment was performed for all retained studies following application of the inclusion and exclusion criteria. Using this method, studies deemed be of low quality would not be excluded from the systematic review, but would be retained, and their methodological strengths and weaknesses discussed where relevant. To define an overall study quality rating for each included study, validated study-type specific tools were used to assess the risk of bias, plus additional important quality features were extracted. Specific details for each study type are outlined below.

Systematic Reviews (SRs) and Meta-analyses (MAs)
- The following questions were assessed as per the Assessing the Methodological Quality of Systematic Reviews (AMSTAR)[2] tool using yes, no, or unclear:
  1. Was an 'a priori' design provided?
  2. Was there duplicate study selection and data extraction?
  3. Was a comprehensive literature search performed?
  4. Was the status of publication (i.e., grey literature) used as an inclusion criterion?
  5. Was a list of studies (included and excluded) provided?
  6. Were the characteristics of the included studies provided?
  7. Was the scientific quality of the included studies assessed and documented?
  8. Was the scientific quality of the included studies used appropriately in formulating conclusions?
  9. Were the methods used to combine the findings of studies appropriate?
  10. Was the likelihood of publication bias assessed?
  11. Was the conflict of interest included?
- Additional assessed items included and were assessed as yes, no, or unclear:
  1. Reporting of funding sources.

Genome Sequencing Studies and Retrospective Cohort Studies (RCS)
- The following domains were assessed using the Risk of Bias in Non-Randomized Studies – of Intervention (ROBINS-I)[3] tool using low risk, moderate risk, serious risk, critical risk, or unclear:
  1. Confounding
  2. Patient selection (selection bias)
  3. Intervention classification (performance bias)
  4. Deviation from intended intervention (performance bias)
  5. Missing data (reporting bias)
  6. Outcome measurements (detection bias)
  7. Selection of reported outcomes (detection bias)
- Additional assessed items included and were assessed as yes, no, or unclear:
  1. Adequately powered statistical analysis
  2. Reporting of funding sources
  3. Industry funding

Quality Assessment Results

A total of 86 studies identified by our systematic review informed the recommendations. This body of evidence comprised of two systematic reviews with meta-analyses, four genome sequencing studies, two prospective cohort studies, and 78 retrospective cohort studies. In the following sections, the quantity of the evidence as determined by the number of studies that met our inclusion criteria and were retained, the evidence type as determined by study design, the quality of that evidence as determined by the quality assessment, and its consistency are all reported, both as individual studies and in totality, statement by statement. The quality assessment of the genome sequencing studies is included in Supplemental Table 1, the prospective cohort studies in Supplemental Table 2, retrospective cohort studies in Supplemental Table 3, and the systematic reviews and meta-analyses in Supplemental Table 4. The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) Quality of Evidence definitions are presented in Supplemental Table 5. And finally, the GRADE Quality of Evidence for each recommendation/outcome is presented in Supplemental Table 6.

Overall, the body of evidence included in this clinical practice guideline represents a methodologically rigorous and representative summary of the available evidence with quality of evidence that ranges from high to very low.

Assessing the Strength of Recommendations

Development of recommendations required that the panel review the identified evidence and make a series of key judgments:

1. What are the significant findings related to each KQ or outcome? Determine any regulatory requirements and/or evidence that support a specific action.

2. What is the overall quality of evidence supporting each KQ or outcome? Quality of evidence is graded as High, Moderate, Low, and Very Low, based on published criteria (Supplemental Table 5). Quality of evidence is a key element in determining the strength of a recommendation.

3. What is the strength of each recommendation?

4. What is the net balance of benefits and harms? The panel used the Evidence to Decision Framework[4] (EtD) to frame, discuss, and document their decisions for each recommendation.

Strength of Recommendation, Quality Assessment, and Summary of the Benefits and Harms by Guideline Statement

Statement 1. *Strong Recommendation*. – IDH mutational testing must be performed on all diffuse gliomas (DGs).

The strength of evidence to support this guideline statement is *moderate*. Refer to Supplemental Tables 1 and 3 for the risk of bias assessment of the individual studies and Table 6 for the aggregate strength of evidence assessment. The evidence base informing this recommendation comprises 38 studies.[5-42] Four studies were genome sequencing studies,[9-12] while the remaining 34 studies were retrospective cohort studies.[5-8, 13-42] All genome sequencing studies[9-12] obtained samples retrospectively and were assessed as intermediate-low quality based on risk of selection bias, while no other forms of bias were identified. The retrospective cohort studies all suffered from risk of selection bias and were assessed as low[5, 7, 13-16, 19, 22-24, 26-42] and very low quality[6, 8, 17, 18, 20, 21, 25] based on risk of selection bias in addition to risk in performance, detection, and reporting domains. The aggregate risk of bias across the entire evidence base was serious but strength of evidence was up-graded based on a strong association between IDH mutational testing and diffuse glioma diagnosis classification.

Summary of Benefits and Harms of Implementing Recommendation 1

Based on the available evidence and WHO classification of diffuse gliomas, all EP members agree this problem to be a priority (100%, n=9/9), the benefits of testing to outweigh any potential harms (100%, n=7/7), and the recommendation feasible to implement (100%, n=8/8). Although all members agreed that the guidance was feasible to implement, a small minority (14.29%, n=1/7) concluded that not all key stakeholders would find the guidance acceptable. Additionally, the EP members were divided on the magnitude of resources requirements for this guidance with 83.5% (n=5/6) agreeing there would be a moderate cost increase and 16.67% (n=1/6) concluding the increase to be negligible.

Statement 2. *Strong Recommendation*. – ATRX should be assessed in all IDH-mutant DGs unless they show 1p/19q codeletion.

The strength of evidence to support guideline statement 2 is *moderate.* Refer to Supplemental Table 3 for the risk of bias assessment of the individual studies and Table 6 for the aggregate strength of evidence assessment. Recommendation statement 2 was informed by 12 retrospective cohort studies.[14-16, 41-49] Eight of these studies were assessed as low quality,[14-16, 41-43, 47, 48] and four were assessed as very low quality.[44-46, 49] All included studies were limited by a critical risk of selection bias, plus individual studies were further limited by risk of bias in performance,[16, 45, 46] detection,[14, 16, 44-49] and reporting[42, 44-47, 49] domains. Although the aggregate risk of bias across the evidence base was very serious, the evidence was up-graded based on a strong association between ATRX assessment and diffuse glioma WHO classification.

Summary of Benefits and Harms of Implementing Recommendation 2

All EP members agreed that the benefits of ATRX assessment were moderate (50.00%; n=3/6) or large (50.00%; n=3/6), while the harms ranged from large to trivial (large, 40.00%, n=2/5; small, 40.00%, n=2/5; trivial, 20.00%, n=1/5), but all members concluded that the benefits outweighed (80.00%, n=4/5) or probably outweighed (20.00%, n=1/5) the harms. Although 40.00% (n=2/5) of EP members concluded this testing carried a moderate cost, all members agreed that the guidance was acceptable to key stakeholders (100%, n=7/7) and feasible to implement (100%, n=8/8).

Statement 3. *Conditional Recommendation.* – TP53 mutation should be assessed in all IDH-mutant DG unless they show 1p/19q codeletion.
The strength of evidence to support guideline statement 3 is *low*. Refer to Supplemental Tables 1-3 for the risk of bias assessment of the individual studies and Table 6 for the aggregate strength of evidence assessment. Recommendation 3 focuses on the need for TP53 assessment and was informed by two intermediate-low quality genome sequencing study,[10, 11] one intermediate quality prospective cohort study,[50] one intermediate-low quality prospective cohort study,[51] and 15 retrospective cohort dies.[7, 14, 15, 20, 34, 41, 44, 48, 52-58] The retrospective cohort studies were assessed as low[7, 14, 15, 34, 41, 48, 52-54, 56-58] and very low quality[20, 44, 55] based on risk of bias in selection,[7, 14, 15, 20, 34, 41, 44, 48, 52-58] performance,[7, 20, 52] detection,[14, 20, 34, 44, 48, 52-55, 57, 58] and reporting[20, 34, 44, 53, 55-58] domains. The aggregate risk of bias for the evidence base was very serious and evidence was not further down- or up-graded based on any domain.

Summary of Benefits and Harms of Implementing Recommendation 3
Expert Panel members were more divided on the EtD when discussing TP53 mutation assessment. While 50.00% (n=4/8) of members concluded that testing carried large benefits, 25.00% (n=2/8) concluded that the benefits were moderate, and the other 25.00% (n=2/8) concluded that the benefits were small. Similarly, 14.28% (n=1/7) of members concluded that the harms of testing were trivial, while 71.43% (n=5/7) agreed they were small and an additional 14.28% (n=1/7) concluded that the harms were large. When considering the balance of harms and benefits, 20.00% (n=1/5) of members concluded that there was only a balance, while 60.00% (n=3/5) agreed that the benefits probably outweighed the harms, and the final 20.00% (n=1/5) concluded that the benefits to outweigh the harms. Half of the members (50.00%, n=1/2) concluded that addition of this testing would result in a moderate cost increase and the other 50.00% (n=1/2) concluded that the cost would be negligible. Taken together, a majority of EP members agreed that the guidance would probably be acceptable to key stakeholder (66.67%, n=4/6) and feasible to implement (87.50%, n=7/8).

Statement 4. *Strong Recommendation.* – 1p/19q codeletion must be assessed in IDH-mutant DGs unless they show ATRX loss or TP53 mutations.
The strength of evidence to support guideline statement 4 is *moderate.* Refer to Supplemental Tables 1 and 3 for the risk of bias assessment of the individual studies and Table 6 for the aggregate strength of evidence assessment. The evidence base supporting Recommendation 4 comprises one intermediate-low quality genome sequencing study[11] and 11 retrospective cohort studies.[8, 17, 34, 41, 42, 47-49, 55, 59, 60] All retrospective cohort studies carry a critical risk of selection bias, plus individual studies were further limited by risk of bias in performance,[17] detection,[8, 17, 34, 47, 49, 59, 60] and reporting[8, 17, 34, 42, 47, 49, 55, 59, 60] domains. Although the aggregate risk of bias across the evidence base was very serious, the evidence was up-graded based on a strong association between 1p/19q codeletion status and diffuse glioma WHO classification.

Summary of Benefits and Harms of Implementing Recommendation 4
Large agreement was seen across EtD domains when discussing 1p/19q testing. All EP members agreed that the problem is a priority (100%, n=8/8), the benefits to be large (100%, n=9/9) and outweigh any harms (100%, n=6/6), and the guidance to be acceptable to key stakeholders (100%, n=8/8) and feasible to implement (100%, n=9/9).

Statement 5. *Conditional Recommendation.* – CDKN2A homozygous deletion testing may be performed on IDH-mutant diffuse astrocytic gliomas.

The strength of evidence to support this guideline statement is *moderate*. Refer to Supplemental Tables 2 and 3 for the risk of bias assessment of the individual studies and Table 6 for the aggregate strength of evidence assessment. The evidence base informing this statement comprises two prospective cohort studies[50, 51] and nine retrospective cohort studies.[33, 57, 58, 61-66] The included studies were assessed as intermediate,[50] intermediate-low,[51] low[33, 57, 58, 63-66] and very low quality[61, 62] based on risk of bias in selection,[33, 50, 51, 57, 58, 61-66] performance,[51, 61, 63, 66] detection,[33, 50, 57, 58, 61, 62, 64, 66] and reporting[33, 50, 51, 57, 58, 61-66] domains. Additionally, two studies reported statistical analyses that were underpowered,[62, 66] and one did not report on sources of funding.[62] Although the aggregate risk of bias across the evidence base was very serious, the evidence was up-graded based on a strong association between CDKN2A deletion and diffuse glioma WHO classification.

Summary of Benefits and Harms of Implementing Recommendation 5
The EP members were divided on the priority of this issue with 44.44% (n=4/9) agreeing it was a priority but the remaining members deeming it as probably a priority (44.44%, n=4/9) and probably not a priority (11.11%, n=1/9). Based on the identified evidence, a majority of EP members (66.67%, n=4/6) agreed that the benefits of testing for CDKN2A deletion to be large and the harms to be small (66.67%, n=4/6), with all members concluding that the benefits either outweighed the harms (50%, n=2/4) or probably outweighed the harms (50.00%, n=2/4). Additionally, all EP members agreed that the guidance would be acceptable to key stakeholders (yes, 37.50%, n=3/8; probably yes, 62.50%, n=5/8) and feasible to implement (yes, 62.50%, n=5/8; probably yes, 37.50%, n=3/8).

Statement 6. Strong Recommendation. – MGMT promoter methylation testing should be performed on all glioblastoma IDH-wild type (WT).
The strength of evidence to support this guideline statements is *moderate*. Refer to Supplemental Tables 1-4 for the risk of bias assessment of the individual studies and Table 6 for the aggregate strength of evidence assessment. This recommendation statement was informed by two meta-analyses,[67, 68] three genome sequencing studies,[9, 10, 12] and five retrospective cohort studies.[59, 61, 69-71] The included meta-analyses were assessed as high[68] and high-intermediate[67] quality. Both did not report on using publication status as a study selection inclusion criterium,[67, 68] and one did not report on conflict of interest or sources of funding.[67] The genome sequencing studies were all assessed as intermediate-low quality based on retrospective acquisition of samples in all,[9, 10, 12] plus individual moderate risk of reporting[9] and detection bias.[9, 12] Finally, the retrospective cohort studies were assessed as low[59, 69, 70] and very low quality[61, 71] based on risk of bias. The aggregate risk of bias of the evidence base was serious and the evidence was not further up- or down-graded for any domain.

Summary of Benefits and Harms of Implementing Recommendation 6
Based on the available evidence and the association between MGMT promoter methylation and overall survival, EP members agreed that the benefits of testing were moderate (50.00%, n=3/6) or large (50.00%, n=3/6). However, the harms of testing were deemed to range from large (33.33%, n=2/6) through trivial (16.67%, n=1/6), with most members concluding that the harms were large (33.33%, n=2/6) or moderate (33.33%, n=2/6). Despite the range of perceived harms, all EP members agreed that the benefits of testing outweighed the harms (yes, 80.00%, n=4/5; probably yes, 20.00%, n=1/5). Additionally, most EP members concluded that the guidance would be acceptable to key stakeholders (75.00%, n=6/8) and feasible to implement (77.78%, n=7/9). When discussing the impact of this guidance on health equity, while 40.00% (n=2/5) concluded that there would be probably no impact, an additional 40.00% (n=2/5) agreed that equity would bd increased, and the final 20.00% (n=1/5) concluded that it would probably be reduced.

Statement 7. *Conditional Recommendation.* – For IDH-mutant DGs, MGMT promoter methylation testing may not be necessary.
The strength of evidence to support this guideline statements is *low*. Refer to Supplemental Table 1 and 3 for the risk of bias assessment of the individual studies and Table 6 for the

aggregate strength of evidence assessment. The evidence base informing this statement comprises one genome sequencing study[12] and four low quality retrospective cohort studies.[22, 28, 32, 72] The genome sequencing study[12] was assessed as intermediate-low quality based on a serious risk of selection bias and a moderate risk of detection bias. All retrospective cohort studies were limited by critical risk of selection bias.[22, 28, 32, 72] Individuals studies were further limited by risk of bias in performance,[22, 32] reporting,[22, 28, 32, 72] and detection[28, 72] domains, as well as underpowered statistical analyses in two,[22, 72] and a lack of reporting funding sources in one.[32] The aggregate risk of bias across these studies was very serious but evidence was not further down-graded for any domain.

Summary of Benefits and Harms of Implementing Recommendation 7
Based on the available evidence the EP was divided on multiple domains of the EtD. A majority of EP members concluded that this problem was probably not a priority (50.00%, n=3/6), with minorities concluding the problem was not a priority (16.67%, n=1/6) or probably was a priority (33.33%, n=2/6). When considering the benefits and harms of testing MGMT promoter methylation in an IDH-mutant diffuse glioma, 50.00% (n=3/6) of EP members agreed that the benefits were small, while 33.33% (n=2/6) concluded that they were moderate, and 16.67% (n=1/6) concluded that they were large. The perceived harms were thought to range from large (16.67%, n=1/6) down to small (66.67%, n=4/6) and a majority of EP members (60.00%, n=3/5) agreed that there was a balance between the benefits and harms. A minority (40.00%, n=2/5) concluded that the harms of testing outweighed the benefits. Despite this divide, a majority of EP members (83.33%, n=5/6) agreed that this guidance was implementable. When discussing key stakeholders, 50.00% (n=2/4) of members agreed that the guidance would be acceptable, while 25.00% (n=1/4) concluded it would probably be acceptable, and 25.00% (n=1/4) concluded it would probably not be acceptable.

Statement 8. *Conditional Recommendation.* – TERT promoter mutation may be used to provide further support for the diagnosis of oligodendrogliomas and IDH-WT glioblastomas.
The strength of evidence to support this guideline statements is *low*. Refer to Supplemental Tables 1 and 3 for the risk of bias assessment of the individual studies and Table 6 for the aggregate strength of evidence assessment. This recommendation statement was informed by two genome sequencing studies[11, 12] and 11 retrospective cohort studies.[6, 13, 17, 25, 41, 57, 73-77] The genome sequencing studies[11, 12] were assessed as intermediate-low quality while the retrospective cohort studies were assessed as low[13, 41, 57, 73, 74, 76, 77] and very low quality.[6, 17, 25, 75] Included studies suffered from risk of bias in selection,[6, 11-13, 17, 25, 41, 57, 73-77] performance,[6, 17, 25, 73] reporting,[6, 13, 17, 25, 57, 73-75, 77] and detection[12, 13, 17, 25, 57, 73-75, 77] domains. The aggregate risk of bias across these studies was very serious but evidence was not further downgraded for any domain.

Summary of Benefits and Harms of Implementing Recommendation 8
Based on the identified evidence, EP members agreed that the benefits of using TERT promoter methylation to support a diagnosis of oligodendroglioma and IDH wild-type glioblastoma were moderate (33.33%, n=2/6) to large (66.67%, n=4/6), while the harms ranged from large (20.00%, n=1/5) to trivial (40.00%, n=2/5). When considering the balance, most EP members (75.00%, n=3/4) agreed that the benefits outweighed the harms. Although all EP members (100%, n=4/4) noted that this testing would result in a moderate cost, it was agreed that the guidance would be acceptable to key stakeholders (yes, 33.33%, n=2/6; probably yes, 66.67%, n=4/6). However, a minority of EP members (20.00%, n=1/5) concluded that this guidance was probably not feasible to implement. Most members agreed that the guidance was either feasible (60.00%, n=3/5) or probably feasible (20.00%, n=1/5).

Statement 9. *Strong Recommendation.* – For histologic grade II-III DGs that are IDH-WT, testing should be performed for whole chromosome 7 gain/whole chromosome 10 loss, EGFR amplification, and TERT promoter mutation to establish the molecular diagnosis of glioblastoma, grade 4.

The strength of evidence to support this guideline statements is *moderate*. Refer to Supplemental Tables 1 and 3 for the risk of bias assessment of the individual studies and Table 6 for the aggregate strength of evidence assessment. The evidence base informing this statement includes studies evaluating testing of chromosome 7, chromosome 10, EGFR and TERT. One genome sequencing study[9] and one retrospective cohort study[61] comprise the evidence base for chromosome 7, one retrospective cohort study was included for chromosome 10,[61] two genome sequencing studies[9, 10] and eight retrospective cohort studies[6, 22, 41, 61, 78-81] for EGFR, and one genome sequencing study[12] and eight retrospective cohort studies for TERT.[6, 13, 17, 41, 73-76] Included studies were assessed as intermediate-low through very low quality and suffered from risk of bias in selection,[6, 9, 10, 12, 13, 17, 22, 41, 61, 73-76, 78-81] performance,[6, 17, 22, 61, 73, 81] reporting,[6, 9, 13, 17, 22, 61, 73-75, 78, 79, 81] and detection[9, 12, 13, 17, 61, 73-75, 78-80] domains. Strength of evidence was assessed for each target individually and overall, for the statement. For both chromosome 7 and EGFR testing, the aggregate risk of bias for studies included in the evidence base was serious and evidence was not further downgraded, resulted in moderate strength of evidence. For chromosome 10 the risk of bias of the one included study was very serious, this carrying a low strength of evidence. Finally, the strength of evidence for TERT was also low based on a very serious aggregate risk of bias and no further downgrading of strength. The overall statement was assessment as moderate.

Summary of Benefits and Harms of Implementing Recommendation 9
Based on the identified evidence for all four included targets, EP members concluded that the benefits of testing to ranged from moderate (11.11%, n=1/9) to large (88.89%, n=8/9), and the harms to range from large (16.67%, n=1/6) to trivial (50.00%, n=5/6). All EP members agreed that the benefits either outweighed the harms (83.33%, n=5/6) or probably outweighed the harms (16.67%, n=1/6). All EP members (100%, 5/5) also agreed that testing would carry a moderate cost but most members concluded that testing would increase health equity (increase, 20.00%, n=1/5; probably increase, 60.00%, n=3/5). The guidance is deemed to be acceptable to key stakeholders (yes, 83.33%, n=5/6; probably yes, 16.67%, n=1/6) and feasible to implement (yes, 83.33%, n=5/6; probably yes, 16.67%, n=1/6).

Statement 10. *Strong Recommendation.* – H3 K27M testing must be performed in diffuse gliomas that involve the midline in the appropriate clinical and pathologic setting.
The strength of evidence to support this guideline statements is *moderate*. Refer to Supplemental Table 3 for the risk of bias assessment of the individual studies and Table 6 for the aggregate strength of evidence assessment. This recommendation statement was informed by two retrospective cohort studies.[82, 83] Both retrospective studies were limited by risk of bias in selection, performance, reporting, and detection domains.[82, 83] In addition, one study reported underpowered statistical analyses.[82] The aggregate risk of bias for the evidence base was very serious; however, the strength of evidence was up-graded based on a strong association between H3 K27M testing and diffuse glioma diagnosis using WHO classifications.

Summary of Benefits and Harms of Implementing Recommendation 10
Based on the identified evidence, most EP members agreed that H3 K27M testing carried large benefits (88.89%, n=8/9). However, the EP members were divided on the magnitude of the harms with 75.00% (n=6/8) of members believing them to be small (25.00%, n=2/8) or trivial (50.00%, n=4/8), but 25.00% (n=2/8) concluding that the harms were large. Despite this divide, all EP members (100%, n=7/7) agreed that the benefits outweighed the harms. All EP members agreed that the guidance would be acceptable to key stakeholders (yes, 75.00%, n=6/8; probably yes, 25.00%, n=2/8) and feasible to implement (yes, 75.00%, n=6/8; probably yes, 25.00%, n=2/8).

Statement 11. *Conditional Recommendation.* – H3 G34 testing may be performed in children and young adults with IDH-WT DGs.
The strength of evidence to support this guideline statements is *low*. Refer to Supplemental Table 3 for the risk of bias assessment of the individual studies and Table 6 for the aggregate strength of evidence assessment. The evidence base informing this statement comprises two

low quality retrospective cohort studies.[26, 84] In addition to suffering from critical risk of selection bias, both studies were limited by a moderate risk of reporting bias,[26, 84] and one was also limited by risk of performance and detection bias.[26] The aggregate risk of bias for the studies was very serious and the evidence was not further down-graded for any domain.

Summary of Benefits and Harms of Implementing Recommendation 11
Based on the identified evidence, EP members agreed that the problem addressed with this guidance statement was a priority (yes, 44.44%, n=4/9; probably yes, 55.56%, n=5/9). When considering the benefits and harms, the magnitude of the benefits ranged from moderate (37.50%, n=3/8) to large (62.50%, n=5/8) and the harms ranged from moderate (1.28%, n=1/7) to trivial (28.57%, n=2/7) with all EP members concluding that the benefits outweighed the harms (yes, 57.14%, n=4/7; probably yes, 42.86%, n=3/7). Although most EP members (55.56%, n=5/9) agreed that H3 G34 testing would carry a moderate cost, the majority also agreed that the testing would increase health equity (increase, 40.00%, n=2/5; probably increase, 40.00%, n=2/5). All EP members agreed that this guidance would be acceptable to key stakeholders (yes, 57.14%, n=4/7; probably yes, 48.86%, n=3/7) and feasible to implement (yes, 57.14%, n=4/7; probably yes, 42.86%, n=3/7).

Statement 12. *Strong Recommendation.* – BRAF mutation testing (V600) may be performed in DGs that are IDH-WT and H3-WT.
The strength of evidence to support this guideline statements is *low*. Refer to Supplemental Tables 2 and 3 for the risk of bias assessment of the individual studies and Table 6 for the aggregate strength of evidence assessment. The evidence base informing this statement is comprised of one intermediate quality prospective cohort study[50] and four retrospective cohort studies.[6, 85-87] Two included retrospective studies were assessed as very low quality based on serious or critical risk of bias across more than one domain.[6, 85] The other two retrospective studies were also assessed as very low quality and this was based on critical risk of selection bias, moderate risk of bias in other domains, underpowered statistical analyses, and a lack of funding source reporting.[86, 87] The aggregate risk of bias for the studies was very serious but evidence was not further down-graded for any domain.

Summary of Benefits and Harms of Implementing Recommendation 12
Although the strength of evidence supporting this statement is low, the EP has elected to propose a strong recommendation for BRAF mutation testing. Through a review of the identified evidence base and discussion using the EtD, the benefits of this testing outweighed the perceived harms (yes, 66.67%, n=4/6; probably yes, 33.33%, n=2/6). Further to this, all EP members agreed that patients would experience harms involving a different treatment protocol if the opposite guidance were provided and this provides the basis for a strong statement. All EP members agreed that this guidance would be acceptable to key stakeholders (yes, 71.43%, n=5/7; probably yes, 28.57%, n=2/7) and feasible to implement (yes, 71.43%, n=5/7; probably yes, 28.57%, n=2/7).

Statement 13. *Conditional Recommendation.* – MYB/MYBL1 and FGFR1 testing may be performed in children and young adults with DG that are histologic grade II-III and are IDH-WT and H3-WT.
The strength of evidence to support this guideline statements is *low*. Refer to Supplemental Table 3 for the risk of bias assessment of the individual studies and Table 6 for the aggregate strength of evidence assessment. The evidence base informing this statement includes three retrospective cohort studies evaluating MYB testing[6, 88, 89] and one retrospective cohort study evaluating FGFR1 testing.[90] The four studies were assessed as low[88, 89] and very low quality[6, 90] based on risk of bias in selection,[6, 88-90] performance,[6] reporting,[6, 88-90] and detection[88-90] domains. Strength of evidence was assessed for each target individually and then overall for the entire statement. The aggregate risk of bias for MYB studies was very serious but evidence was not further downgraded for any domain. The risk of bias for the one FGFR1 study was very serious. As only one study was identified, strength of evidence is dependent only on the risk of bias. The strength of evidence for the entire statement was assessed as low.

Summary of Benefits and Harms of Implementing Recommendation 13
The EP provided a range of EtD responses when discussing this statement. Based on the identified evidence, the magnitude of benefits for this testing ranged from small (20.00%, n=1/5) through large (20.00%, n=1/5) while the harms were considered to be small (50.00%, n=2/4) or trivial (50.00%, n=2/4). Half of the EP members (50.00%, n=3/6) concluded that the benefits outweighed the harms and half concluded that the benefits maybe outweighed the harms (50.00%, n=3/6). Although all EP members agreed that the guidance would be acceptable to key stakeholders (yes, 66.67%, n=4/6; probably yes, 33.33%, n=2/6), and the majority agreed that the guidance would be feasible (yes, 50.00%, n=3/6; probably yes, 33.33%, n=2/6), a minority (16.67%, n=1/6) concluded that the guidance was probably not feasible to implement.

Good Practice Statements
According to the GRADE approach, good practice statements (GPS) are recommendations panels may consider important but are not appropriate to be formally rated for quality of evidence.[91] During the period of the systematic literature review and drafting of recommendations, several clinically relevant investigations were published on the topic of biomarker testing for diffuse glioma subtypes not originally covered by the Patient/population, Intervention, Comparator, and Outcome (PICO) frameworks. To include the emerging evidence and in anticipation of the WHO update, the EP wanted to address the following questions:

1. What genetic and molecular alterations should be included for optimal classification of bithalamic glioma?
2. What genetic and molecular alterations should be included for optimal classification of infantile-type hemisphere glioma?
3. What genetic and molecular alterations should be included for optimal classification of diffuse pediatric-type high grade glioma, H3-wildtype, IDH-wildtype?

A targeted literature search based on the DG subtypes and alterations of interest was designed for these questions. For bithalamic gliomas, the EP was interested in EGFR mutations, for infantile-type hemisphere gliomas, ALK, ROS1, NTRK, and MET alterations were considered, and finally, for diffuse pediatric-type high grade glioma, EGFR, PDGFRA, and MYCN amplifications were of interest. The EP chair reviewed the available literature and drafted statements which then underwent consensus voting by all members of the EP. The EP believes that the tests included in the GPS will provide prognostic or treatment-related information for clinicians to consider. Future updates to the guideline will address these tests using a formal rating of the evidence and will include a grade for the strength of recommendation.

Dissemination Plans
The CAP hosts a Biomarkers for the Diagnostic Testing for Diffuse Gliomas guideline web page which will include a link to the manuscript and supplement; a summary of the recommendations, a PowerPoint slide deck (Microsoft Corporation, Redmond, WA), a frequently asked question (FAQ) document, and an infographic. The guideline will be promoted and presented at various society meetings.

Supplemental Table 1. Quality Assessment of Included Genome Sequencing Studies

Study		Brennan 2013[9]	McClendon 2008[10]	Brat 2015[11]	Ceccarelli 2016[12]
ROBINS-I Assessment	Confounding	MR	MR	MR	MR
	Patient selection	SR	SR	SR	SR
	Intervention classification	LR	LR	LR	LR
	Deviation from intended intervention	LR	LR	LR	LR
	Missing data	MR	LR	LR	LR

Supplemental Table 1. Quality Assessment of Included Genome Sequencing Studies				
Study		Brennan 2013[9]	McClendon 2008[10]	Brat 2015[11]	Ceccarelli 2016[12]
	Outcome measurements	LR	LR	LR	LR
	Selection of reported outcomes	MR	LR	LR	MR
	Overall Risk of Bias	SR	SR	SR	SR
Adequately powered		Y	Y	Y	Y
Reported funding sources		Y	Y	Y	Y
Industry funded		N	N	N	N

Abbreviations: LR, low risk; MR, moderate risk; SR, serious risk; N, no; Y, yes.

Supplemental Table 2. Quality Assessment of Included Prospective Cohort Studies

Study		Jonsson 2019[50]	Pessoa 2019[51]
ROBINS-I Assessment	Confounding	MR	MR
	Patient selection	MR	SR
	Intervention classification	LR	MR
	Deviation from intended intervention	LR	MR
	Missing data	MR	MR
	Outcome measurements	MR	LR
	Selection of reported outcomes	MR	LR
	Overall Risk of Bias	MR	SR
Adequately powered		Y	N
Reported funding sources		Y	Y
Industry funded		N	N

Abbreviations: CR, critical risk; LR, low risk; MR, moderate risk; SR, serious risk; N, no; NS, no statistical analysis; U, unclear/unsure; Y, yes.

Supplemental Table 3. Quality Assessment of Included Retrospective Cohort Studies

Study		Ahmadi 2012[5]	Aibaidula 2017[6]	Alentorn 2014[7]	Bady 2012[69]	Boots-Sprenger 2013[8]	Brandes 2017[70]	Cai 2014[43]	Castel 2015[82]	Chan 2015[13]	Chan 2015[78]
ROBINS-I Assessment	Confounding	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR
	Patient selection	CR	CR	CR	CR	CR	CR	CR	CR	CR	CR
	Intervention classification	LR	LR	LR	LR	LR	LR	LR	LR	LR	LR
	Deviation from intended intervention	LR	MR	MR	LR	LR	MR	LR	SR	LR	LR
	Missing data	MR	SR	MR	LR	CR	MR	LR	MR	MR	MR
	Outcome measurements	LR	LR	LR	MR	MR	LR	LR	MR	MR	MR
	Selection of reported outcomes	LR	LR	LR	MR	MR	LR	LR	MR	LR	MR
	Overall Risk of Bias	CR	CR	CR	CR	CR	CR	CR	CR	CR	CR
Adequately powered		Y	Y	Y	Y	NS	Y	Y	NS	Y	Y
Reported funding sources		Y	Y	Y	Y	N	Y	Y	Y	Y	Y
Industry funded		N	N	N	N	U	N	N	N	N	N

Abbreviations: CR, critical risk; LR, low risk; MR, moderate risk; SR, serious risk; N, no; NS, no statistical analysis; U, unclear/unsure; Y, yes.

Supplemental Table 3. Quality Assessment of Included Retrospective Cohort Studies (continued)

Study		Chaurasia 2016[14]	Collins 2014[61]	Cryan 2014[44]	Dubbink 2016[15]	Ebrahimi 2016[16]	Eckel-Passow 2015[17]	Felsberg 2010[18]	Figarella-Branger 2012[52]	Fontana 2016[19]
ROBINS-I Assessment	Confounding	MR	MR	MR	MR	MR	MR	MR	MR	MR

Supplemental Table 3. Quality Assessment of Included Retrospective Cohort Studies (continued)										
Study		Chaurasia 2016[14]	Collins 2014[61]	Cryan 2014[44]	Dubbink 2016[15]	Ebrahimi 2016[16]	Eckel-Passow 2015[17]	Felsberg 2010[18]	Figarella-Branger 2012[52]	Fontana 2016[19]
	Patient selection	CR	CR	CR	CR	CR	CR	CR	CR	CR
	Intervention classification	LR	LR	LR	LR	LR	MR	LR	LR	LR
	Deviation from intended intervention	LR	SR	LR	LR	MR	MR	LR	MR	LR
	Missing data	LR	MR	CR	LR	MR	CR	SR	MR	MR
	Outcome measurements	MR	LR	MR	LR	MR	MR	SR	MR	MR
	Selection of reported outcomes	LR	MR	MR	LR	LR	MR	LR	MR	MR
	Overall Risk of Bias	CR	CR	CR	CR	CR	CR	CR	CR	CR
Adequately powered		Y	Y	N	Y	Y	Y	Y	NS	Y
Reported funding sources		Y	Y	N	Y	Y	Y	N	N	Y
Industry funded		N	N	U	N	N	N	U	U	N

Abbreviations: CR, critical risk; LR, low risk; MR, moderate risk; SR, serious risk; N, no; NS, no statistical analysis; U, unclear/unsure; Y, yes.

Supplemental Table 3. Quality Assessment of Included Retrospective Cohort Studies (continued)											
Study			Gao 2016[73]	Gessi 2013[84]	Gorovets 2012[53]	Gravendeel 2010[20]	Guillaudeau 2009[79]	Hartmann 2013[59]	Hattori 2016[21]	Hewer 2016[45]	Megova 2017[22]	Ikemura 2016[46]
ROBINS-I Assessment	Confounding	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR
	Patient selection	CR	CR	CR	CR	CR	CR	CR	CR	CR	CR
	Intervention classification	LR	LR	LR	MR	LR	LR	MR	LR	LR	SR
	Deviation from intended intervention	MR	LR	LR	LR	LR	LR	LR	MR	MR	LR
	Missing data	MR	MR	MR	SE	MR	MR	CR	SR	MR	MR
	Outcome measurements	MR	LR	MR	MR	MR	MR	MR	MR	LR	MR

Supplemental Table 3. Quality Assessment of Included Retrospective Cohort Studies (continued)										
Study		Gao 2016[73]	Gessi 2013[84]	Gorovets 2012[53]	Gravendeel 2010[20]	Guillaudeau 2009[79]	Hartmann 2013[59]	Hattori 2016[21]	Hewer 2016[45]	Megova 2017[22]	Ikemura 2016[46]
	Selection of reported outcomes	MR	LR	LR	LR	MR	MR	MR	MR	LR	SR
	Overall Risk of Bias	CR	CR	CR	CR	CR	CR	CR	CR	CR	CR
Adequately powered		Y	NS	Y	Y	NS	Y	NS	NS	N	Y
Reported funding sources		Y	N	Y	N	N	Y	Y	N	Y	N
Industry funded		N	U	N	U	U	N	N	U	N	U

Abbreviations: CR, critical risk; LR, low risk; MR, moderate risk; SR, serious risk; N, no; NS, no statistical analysis; U, unclear/unsure; Y, yes.

Supplemental Table 3. Quality Assessment of Included Retrospective Cohort Studies (continued)										
Study		Jha 2011[23]	Juratli 2012[24]	Khuong-Quang 2012[83]	Killela 2014[25]	Kleinshmidt-DeMasters 2013[85]	Korshunov 2017[86]	Korshunov 2015[26]	Labussiere 2014[74]	Labussiere 2010[27]
ROBINS-I Assessment	Confounding	MR	MR	MR	MR	MR	MR	MR	MR	MR
	Patient selection	CR	CR	CR	CR	CR	CR	CR	CR	CR
	Intervention classification	LR	LR	LR	LR	LR	LR	MR	LR	LR
	Deviation from intended intervention	MR	LR	SR	MR	LR	LR	LR	LR	LR
	Missing data	MR	LR	MR	SR	SR	MR	MR	MR	MR
	Outcome measurements	LR	LR	LR	MR	LR	MR	LR	MR	MR
	Selection of reported outcomes	LR	LR	MR	MR	SR	LR	MR	MR	MR
	Overall Risk of Bias	CR	CR	CR	CR	CR	CR	CR	CR	CR
Adequately powered		NS	NS	Y	Y	N	N	Y	Y	Y
Reported funding sources		N	N	Y	Y	Y	N	Y	Y	Y
Industry funded		U	U	N	N	N	U	N	N	N

Abbreviations: CR, critical risk; LR, low risk; MR, moderate risk; SR, serious risk; N, no; NS, no statistical analysis; U, unclear/unsure; Y, yes.

Supplemental Table 3. Quality Assessment of Included Retrospective Cohort Studies (continued)

Study		Lee 2017[28]	Lewandowska 2014[29]	Li 2015[30]	Little 2012[80]	Mellai 2017[60]	Mellai 2011[31]	Minniti 2014[32]	Molenaar 2014[33]	Mukasa 2012[34]
ROBINS-I Assessment	Confounding	MR	MR	MR	MR	MR	MR	MR	MR	MR
	Patient selection	CR	CR	CR	CR	CR	CR	CR	CR	CR
	Intervention classification	LR	LR	LR	LR	LR	LR	LR	LR	LR
	Deviation from intended intervention	LR	LR	LR	LR	LR	MR	MR	LR	LR
	Missing data	SR	SR	MR	LR	MR	MR	MR	MR	MR
	Outcome measurements	MR	MR	LR	LR	MR	LR	LR	MR	MR
	Selection of reported outcomes	MR	MR	SR	MR	MR	MR	LR	LR	MR
	Overall Risk of Bias	CR	CR	CR	CR	CR	CR	CR	CR	CR
Adequately powered		Y	N	Y	NS	NS	Y	Y	Y	Y
Reported funding sources		Y	Y	Y	Y	Y	Y	N	Y	Y
Industry funded		N	N	N	N	N	N	U	N	N

Abbreviations: CR, critical risk; LR, low risk; MR, moderate risk; SR, serious risk; N, no; NS, no statistical analysis; U, unclear/unsure; Y, yes.

Supplemental Table 3. Quality Assessment of Included Retrospective Cohort Studies (continued)

Study		Mulholland 2012[72]	Nakajima 2017[87]	Nobusawa 2009[35]	Nonoguchi 2013[75]	Olar 2015[36]	Polivka 2014[37]	Purkait 2013[62]	Qaddoumi 2016[90]	Qi 2011[38]
ROBINS-I Assessment	Confounding	MR	MR	MR	MR	MR	MR	MR	MR	MR
	Patient selection	CR	CR	CR	CR	CR	CR	CR	CR	CR
	Intervention classification	LR	LR	LR	LR	MR	LR	LR	LR	MR
	Deviation from intended intervention	LR	LR	LR	LR	LR	LR	LR	LR	LR
	Missing data	MR	MR	SR	MR	MR	MR	SR	SR	SR
	Outcome measurements	MR	MR	MR	MR	LR	LR	MR	MR	LR

Supplemental Table 3. Quality Assessment of Included Retrospective Cohort Studies (continued)										
Study		Mulholland 2012[72]	Nakajima 2017[87]	Nobusawa 2009[35]	Nonoguchi 2013[75]	Olar 2015[36]	Polivka 2014[37]	Purkait 2013[62]	Qaddoumi 2016[90]	Qi 2011[38]
	Selection of reported outcomes	MR	MR	MR	MR	MR	LR	SR	MR	LR
	Overall Risk of Bias	CR	CR	CR	CR	CR	CR	CR	CR	CR
Adequately powered		N	N	Y	N	Y	N	N	Y	N
Reported funding sources		Y	N	Y	N	Y	Y	N	Y	Y
Industry funded		N	U	N	U	N	N	U	N	N

Abbreviations: CR, critical risk; LR, low risk; MR, moderate risk; SR, serious risk; N, no; NS, no statistical analysis; U, unclear/unsure; Y, yes.

Supplemental Table 3. Quality Assessment of Included Retrospective Cohort Studies (continued)										
Study		Rajmohan 2016[47]	Reifenberger 2012[71]	Ruano 2009[54]	Sahm 2014[55]	Sanson 2009[3]	Shao 2016[48]	Shibahara 2012[40]	Tatevossian 2010[88]	Wiestler 2013[49]
ROBINS-I Assessment	Confounding	MR	MR	MR	SR	MR	MR	MR	MR	MR
	Patient selection	CR	CR	CR	CR	CR	CR	CR	CR	CR
	Intervention classification	LR	LR	LR	LR	LR	LR	LR	LR	LR
	Deviation from intended intervention	LR	SR	LR	LR	MR	LR	LR	LR	LR
	Missing data	MR	SR	LR	MR	MR	LR	LR	MR	SR
	Outcome measurements	MR	MR	MR	LR	LR	MR	MR	MR	MR
	Selection of reported outcomes	MR	MR	MR	MR	MR	MR	LR	LR	MR
	Overall Risk of Bias	CR	CR	CR	CR	CR	CR	CR	CR	CR
Adequately powered		Y	Y	Y	NS	Y	Y	Y	Y	Y
Reported funding sources		Y	Y	Y	Y	Y	Y	Y	Y	Y
Industry funded		N	N	N	N	N	N	N	N	N

Abbreviations: CR, critical risk; LR, low risk; MR, moderate risk; SR, serious risk; N, no; Y, yes.

Supplemental Table 3. Quality Assessment of Included Retrospective Cohort Studies (continued)										
Study		Wang 2016[56]	Reis 2015[63]	Ramkissoon 2013[89]	Bienkowski 2018[41]	Kim 2018[76]	Rajeswarie 2018[42]	Lee 2018[81]	Shirahata 2018[64]	Higa 2020[57]
ROBINS-I Assessment	Confounding	MR	MR	MR	MR	MR	MR	MR	MR	MR
	Patient selection	CR	CR	CR	CR	CR	CR	CR	CR	CR
	Intervention classification	LR	LR	LR	LR	LR	LR	MR	LR	LR
	Deviation from intended intervention	LR	MR	LR	LR	LR	MR	LR	LR	LR
	Missing data	CR	MR	MR	LR	LR	LR	MR	MR	MR
	Outcome measurements	LR	LR	MR	LR	LR	LR	LR	LR	MR
	Selection of reported outcomes	LR	LR	MR	LR	LR	MR	LR	MR	MR
	Overall Risk of Bias	CR	CR	CR	CR	CR	CR	CR	CR	CR
Adequately powered		Y	Y	N	NS	N	NS	NS	Y	Y
Reported funding sources		Y	Y	Y	Y	Y	N	N	Y	N
Industry funded		N	N	N	N	N	U	U	N	U

Abbreviations: CR, critical risk; LR, low risk; MR, moderate risk; N, no; NS, no statistical analysis; U, unclear/unsure; Y, yes.

Supplemental Table 3. Quality Assessment of Included Retrospective Cohort Studies (continued)					
Study		Yang 2020[58]	Appay 2019[65]	Mirchia 2019[66]	Brito 2019[77]
ROBINS-I Assessment	Confounding	MR	MR	MR	MR
	Patient selection	CR	CR	CR	CR
	Intervention classification	LR	LR	MR	LR
	Deviation from intended intervention	LR	LR	MR	LR
	Missing data	MR	MR	MR	MR
	Outcome measurements	MR	LR	MR	MR
	Selection of reported outcomes	LR	LR	MR	LR
	Overall Risk of Bias	CR	CR	CR	CR
Adequately powered		Y	Y	N	N
Reported funding sources		Y	Y	Y	Y
Industry funded		N	N	N	N

Abbreviations: CR, critical risk; LR, low risk; MR, moderate risk; N, no; NS, no statistical analysis; U, unclear/unsure; Y, yes.

Supplemental Table 4. Quality Assessment of Included Systematic Reviews/Meta-analysis			
Study		Chen 2013[67]	Yin 2014[68]
AMSTAR Assessment	A priori design	Y	Y
	Duplicate study selection and data extraction	Y	Y
	Comprehensive literature search	Y	Y
	Publication status as inclusion criterion	N	N
	List of included and excluded studies	Y	Y
	Characteristics of included studies	Y	Y
	Study quality assessment conducted	Y	Y
	Quality assessment used in formulating conclusions	Y	Y
	Appropriate methods to combine findings	Y	Y
	Publication bias assessment	Y	Y
	Conflict of interest reported	N	Y
Reported funding sources		N	Y

Abbreviations: Int, Intermediate; N, no; Y, yes.

Supplemental Table 5: Quality of Evidence

Designation	Description
High	There is high confidence that available evidence reflects true effect. Further research is very unlikely to change the confidence in the estimate of effect. Included studies will be of high or intermediate quality.
Moderate	There is moderate confidence that available evidence reflects true effect. Further research is likely to have an important impact on the confidence in estimate of effect and may change the estimate. Included studies will be of intermediate or low quality.
Low	There is limited confidence in the estimate of effect. The true effect may be substantially different from the estimate of the effect. Included studies will be of low quality.

Very Low	There is very little confidence in the estimate of effect. The true effect is likely to be substantially different from the estimate of effect. Any estimate of effect is very uncertain. Included studies will be of low or very low quality.

Data derived from Grading of Recommendations Assessment, Development and Evaluation (GRADE) Working Group Materials.[92]

Supplemental Table 6. GRADE Quality of Evidence Assessment

Number of Studies and Design per Outcome (outcome rating[a])	Aggregate Risk of Bias	Inconsistency	Indirectness	Imprecision	Other[b]	Quality of Evidence Grade for Outcome	Overall Quality of Evidence Grade for Statement
Statement 1							
Accurate Diagnosis (critical) 4 GSS, 34 RCS	Serious	Not serious	Not serious	Not serious	Strong association	Moderate	Moderate
Mutation Status (important) 4 GSS, 34 RCS	Serious	Not serious	Not serious	Not serious	Strong association	Moderate	
Overall Survival (critical) 24 RCS	Very serious	Not serious	Not serious	Not serious	None	Low	
Statement 2							
Accurate Diagnosis (critical) 12 RCS	Very serious	Not serious	Not serious	Not serious	Strong association	Moderate	Moderate
Mutation Status (important) 12 RCS	Very serious	Not serious	Not serious	Not serious	Strong association	Moderate	
Overall Survival (critical) 4 RCS	Very serious	Not serious	Not serious	Not serious	None	Low	
Statement 3							
Accurate Diagnosis (critical) 14 RCS	Very serious	Not serious	Not serious	Not serious	None	Low	Low
Mutation Status (important)	Serious	Not serious	Not serious	Not serious	None	Moderate	

Number of Studies and Design per Outcome (outcome rating[a])	Aggregate Risk of Bias	Inconsistency	Indirectness	Imprecision	Other[b]	Quality of Evidence Grade for Outcome	Overall Quality of Evidence Grade for Statement
Supplemental Table 6. GRADE Quality of Evidence Assessment							
2 GSS, 2 PCS, 15 RCS							
Overall Survival (critical) 4 RCS	Very serious	Not serious	Not serious	Not serious	None	Low	
Statement 4							
Accurate Diagnosis (critical) 1 GSS, 11 RCS	Very serious	Not serious	Not serious	Not serious	Strong Association	Moderate	Moderate
Mutation Status (important) 1 GSS 11 RCS	Very serious	Not serious	Not serious	Not serious	Strong association	Moderate	
Overall Survival (critical) 2 RCS	Very serious	Not serious	Not serious	Not serious	None	Low	
Statement 5							
Accurate Diagnosis (critical) 2 PCS, 2 RCS	Very serious	Not serious	Not serious	Not serious	Strong association	Moderate	Moderate
Mutation Status (important) 2 PCS, 8 RCS	Very serious	Not serious	Not serious	Not serious	Strong association	Moderate	
Overall Survival (critical) 4 RCS	Very serious	Not serious	Not serious	Not serious	None	Low	
Statement 6							
Accurate Diagnosis (critical) 1 MA, 2 GSS, 5 RCS	Serious	Not serious	Not serious	Not serious	None	Moderate	Moderate
Mutation Status (important)	Serious	Not serious	Not serious	Not serious	None	Moderate	

Supplemental Table 6. GRADE Quality of Evidence Assessment

Number of Studies and Design per Outcome (outcome rating[a])	Aggregate Risk of Bias	Inconsistency	Indirectness	Imprecision	Other[b]	Quality of Evidence Grade for Outcome	Overall Quality of Evidence Grade for Statement
1 MA, 3 GSS, 4 RCS							
Overall Survival (critical) 2 MA, 3 RCS	Serious	Not serious	Not serious	Not serious	None	Moderate	
Sensitivity , Specificity (critical) 1 RCS	Very serious	NA	NA	NA	NA	Low	
Statement 7							
Mutation Status (important) 1 GSS, 4 RCS	Very serious	Not serious	Not serious	Not serious	None	Low	Low
Statement 8							
Accurate Diagnosis (critical) 2 GSS, 11 RCS	Very serious	Not serious	Not serious	Not serious	None	Low	Low
Mutation Status (important) 1 GSS, 11 RCS	Very serious	Not serious	Not serious	Not serious	None	Low	
Overall Survival (critical) 4 RCS	Very serious	Not serious	Not serious	Not serious	None	Low	
Statement 9							
Chr 7 gain Accurate Diagnosis (critical) 1 GSS, 1 RCS	Serious	Not serious	Not serious	Not serious	None	Moderate	Moderate
Chr 7 gain Overall Survival (critical) 1 RCS	Very serious	NA	NA	NA	NA	Low	
Chr 10 loss Mutation Status	Very serious	NA	NA	NA	NA	Low	

Supplemental Table 6. GRADE Quality of Evidence Assessment

Number of Studies and Design per Outcome (outcome rating[a])	Aggregate Risk of Bias	Inconsistency	Indirectness	Imprecision	Other[b]	Quality of Evidence Grade for Outcome	Overall Quality of Evidence Grade for Statement
(important) 1 RCS							
EGFR amp Accurate Diagnosis (critical) 2 GSS, 9 RCS	Serious	Not serious	Not serious	Not serious	None	Moderate	
EGFR amp Overall Survival (critical) 3 RCS	Very serious	Not serious	Not serious	Not serious	None	Low	
TERTp mut Accurate Diagnosis (critical) 1 GSS, 8 RCS	Very serious	Not serious	Not serious	Not serious	None	Low	
Statement 10							
Mutation Status (important) 2 RCS	Very serious	Not serious	Not serious	Not serious	Strong association	Moderate	Moderate
Statement 11							
Mutation Status (important) 2 RCS	Very serious	Not serious	Not serious	Not serious	None	Low	Low
Statement 12							
Mutation Status (important) 1 PCS, 4 RCS	Very serious	Not serious	Not serious	Not serious	None	Low	Low
Overall Survival (critical) 1 RCS	Very serious	NA	NA	NA	NA	Low	
Statement 13							
MYB Mutation status (important)	Very serious	Not serious	Not serious	Not serious	None	Low	Low

Supplemental Table 6. GRADE Quality of Evidence Assessment							
Number of Studies and Design per Outcome (outcome rating[a])	Aggregate Risk of Bias	Inconsistency	Indirectness	Imprecision	Other[b]	Quality of Evidence Grade for Outcome	Overall Quality of Evidence Grade for Statement
3 RCS							
MYB Overall Survival (critical) 1 RCS	Very serious	NA	NA	NA	NA	Low	
FGFR1 Mutation Status (important) 1 RCS	Very serious	NA	NA	NA	NA	Low	

Abbreviations: EGFR, *Epidermal Growth Factor* ; FGFR1, *Fibroblast Growth Factor Receptor;* GSS, genome sequencing studies; MA, meta-analysis; MYB, *MYB proto-oncogene;* NA, not applicable based on one study included for the outcome; OS, overall survival; PCS, prospective cohort study; RCS, retrospective cohort study; TERTp. *Telomerase Reverse Transcriptase promoter mutation*
a Outcomes were rated *a priori* as critical or important for decision making
b Other category includes assessment for detection of publication bias, large effect, and confounding

Supplemental Figure 1:

Ovid MEDLINE Search String:

1   exp astrocytoma/
2   gliosarcoma/
3   oligodendroglioma/
4   glioma/
5   (glioma* or astrocytoma* or glioblastoma* or gliosarcoma* or oligodendroglioma* or oligoastrocytoma* or GBM or
gliomatosis or oligodendroblastoma* or astroglioma*).tw.
6   ((astrocytic or glial) adj3 (tumor* or tumour* or neoplasm* or malignan* or cancer* or sarcoma*)).tw.
7   or/1-6
8   Proto-oncogene proteins B-raf/
9   Isocitrate Dehydrogenase/
10   ATRX protein, human.nm.
11   MGMT protein, human.nm.
12   Genes, p53/
13   Genes,myb/
14   Tumor Suppressor p53 binding protein 1/
15   Tumor Suppressor Protein p53/
16   Receptor, Epidermal Growth Factor/
17   Chromosomes, Human, Pair 19/
18   Chromosomes, Human, Pair 1/
19   Chromosomes, Human, Pair 10/
20   Chromosomes, Human, Pair 7/
21   Receptor, Fibroblast Growth Factor, Type 1/
22   Receptor, Fibroblast Growth Factor, Type 2/
23   Receptor, Fibroblast Growth Factor, Type 3/
24   Receptor, Fibroblast Growth Factor, Type 4/
25   exp Receptors, Platelet-Derived Growth Factor/
26   Proto-Oncogene Proteins c-met/
27   DNA Mismatch Repair/
28   Neurofibromatosis 1/
29   Genes, Neurofibromatosis 1/
30   Microsatellite Instability/
31   Proto-Oncogene Proteins c-mdm2/
32   Cyclin-Dependent Kinase 4/
33   PTEN Phosphohydrolase/
34   ((IDH$ or BRAF$ or ATRX$ or p53 or TP53 or MYB$ or MYB?L$ or p?TERT$ or TERT$ or FGFR$ or EGFR$ or PDGFR$ or C?met$ or CDKN2A$ or MGMT$ or PTEN or NF?1 or MDM?2 or CDK?4) adj3 (mutation$ or mutated or mutant or alteration$ or aberration* or anomaly or anomalies or abnormal* or defect* or error* or status$ or detect$ or identif$)).tw.
35   ((H3$ or k27M$ or histone?3$) adj3 (mutation* or mutated or mutant or methylation$ or alteration$ or status$ or
screen$ or detect$ or identif$ or modif$)).tw.
36   1p?19q.tw.
37   ((chromosom$ or gene$ or germline) adj3 (mutation$ or mutated or mutant or alteration$ or aberration* or anomaly or anomalies or abnormal* or defect* or gain$ or loss$ or deletion or co?deletion)).tw.
38   (microsatellite adj3 (instability or status)).tw.
39   (MSI or MMR or "mismatch repair").tw.
40   or/8-39
41   exp Sequence Analysis/
42   exp Immunohistochemistry/
43   exp In Situ Hybridization/

44   exp Polymerase Chain Reaction/
45   exp Microarray Analysis/
46   exp Gene Expression Profiling/
47   Comparative Genomic Hybridization/
48   Immunoenzyme Techniques/
49   (Immunohistochem$ or IHC).tw.
50   ((Sanger or next?gen$ or RNA$ or DNA$ or MSP$ or "high throughput" or exome or genome or genomic or targeted) adj3 sequenc*).tw.
51   (microarray or microdissection or macrodissection or methylation or pyro?sequencing).tw.
52   ("polymerase chain" or PCR$ or qPCR$ or RT-PCR or RT-MSP).tw.
53   ("in situ hybridization" or FISH).tw.
54   (RNAseq$ or ((methylation or molecular or RNA or DNA or amplif*) adj3 (extract* or probe* or array*))).tw.
55   ((gene$ or molecular or cytogen$ or genomic or mutation$) adj3 (diagnos$ or analys$ or analyz$ or stratification
or assess$ or detect$ or assay$ or platform$ or array* or amplif* or hybridization)).tw.
56   "lab* developed".tw.
57   ("SNP array" or NGS or ARMS or HPLC or DHPLC or LNA or PNA or LCM or LOH or SSCP or MS?MLPA or MLPA or
MRC-Holland or MMR or R132H or CGH$ or arrayCGH$).tw.
58   ((histopathol* or laborator* or molecular or patholog*) adj3 (validat* or diagnos* or test* or method*)).tw.
59   (FFPE or "formalin fixed" or "paraffin embedded").tw.
60   or/41-59
61   7 and 40 and 60
62   limit 61 to (english language and yr="2008 -Current")
63   limit 62 to (case reports or comment or editorial or letter)
64   62 not 63
65   animals/ not humans/
66   64 not 65
67   ("cell line$" or "cell culture$" or "in vitro" or mouse or murine or fish or rat or porcine or mice or dog or horse or animal).ti.
68   66 not 67
69   remove duplicates from 68
70   cell line, tumor/ or cells, cultured/
71   69 not 70

EMBASE Search String:
Query(((((((('astrocytoma'/de OR 'glioblastoma'/de OR 'pilocytic astrocytoma'/de OR 'glioma'/de OR 'gliosarcoma'/de OR 'oligodendroglioma'/de) OR (((astrocyt* OR glial*) NEAR/3 (tumo?r OR neoplasm? OR malignan* OR cancer* OR sarcoma*)):ti,ab)) OR (('pontine glioma'/de) OR (neurogliocytoma*:ti,ab OR neuroglioma*:ti,ab OR neurospongioma*:ti,ab OR glioma*:ti,ab OR astrocytoma*:ti,ab OR glioblastoma*:ti,ab OR ganglioglioma*:ti,ab OR gliosarcoma*:ti,ab OR oligodendroglioma*:ti,ab OR oligoastrocytoma*:ti,ab OR gbm:ti,ab OR gliomatosis:ti,ab OR oligodendroblastoma*:ti,ab OR astroglioma*:ti,ab))) AND (('b raf kinase'/de OR 'isocitrate dehydrogenase'/de OR 'isocitrate dehydrogenase 1'/de OR 'isocitrate dehydrogenase 2'/de OR 'atrx gene'/de OR 'methylated dna protein cysteine methyltransferase'/de OR 'oncogene myb'/de OR 'protein p53'/de OR 'epidermal growth factor receptor'/de OR 'chromosome 10'/de OR 'chromosome 7'/de OR 'chromosome 19q'/de OR 'chromosome, human, pair 19' OR 'chromosome 1p'/de OR 'chromosome, human, pair 1' OR 'fibroblast growth factor receptor 1'/de OR 'fibroblast growth factor receptor 2'/de OR 'fibroblast growth factor receptor 3'/de OR 'fibroblast growth factor receptor 4'/de OR 'platelet derived growth factor beta receptor'/de OR 'platelet derived growth factor alpha receptor'/de OR 'platelet derived growth factor receptor'/de OR 'scatter factor receptor'/de OR 'mismatch repair'/de OR 'gene expression regulation'/de OR 'neurofibromatosis 1 gene'/de OR 'microsatellite instability'/de OR 'protein mdm2'/de OR 'cyclin dependent kinase 4'/de OR 'histone h3'/de) OR ('tumor suppressor gene'/de) OR (((idh* OR
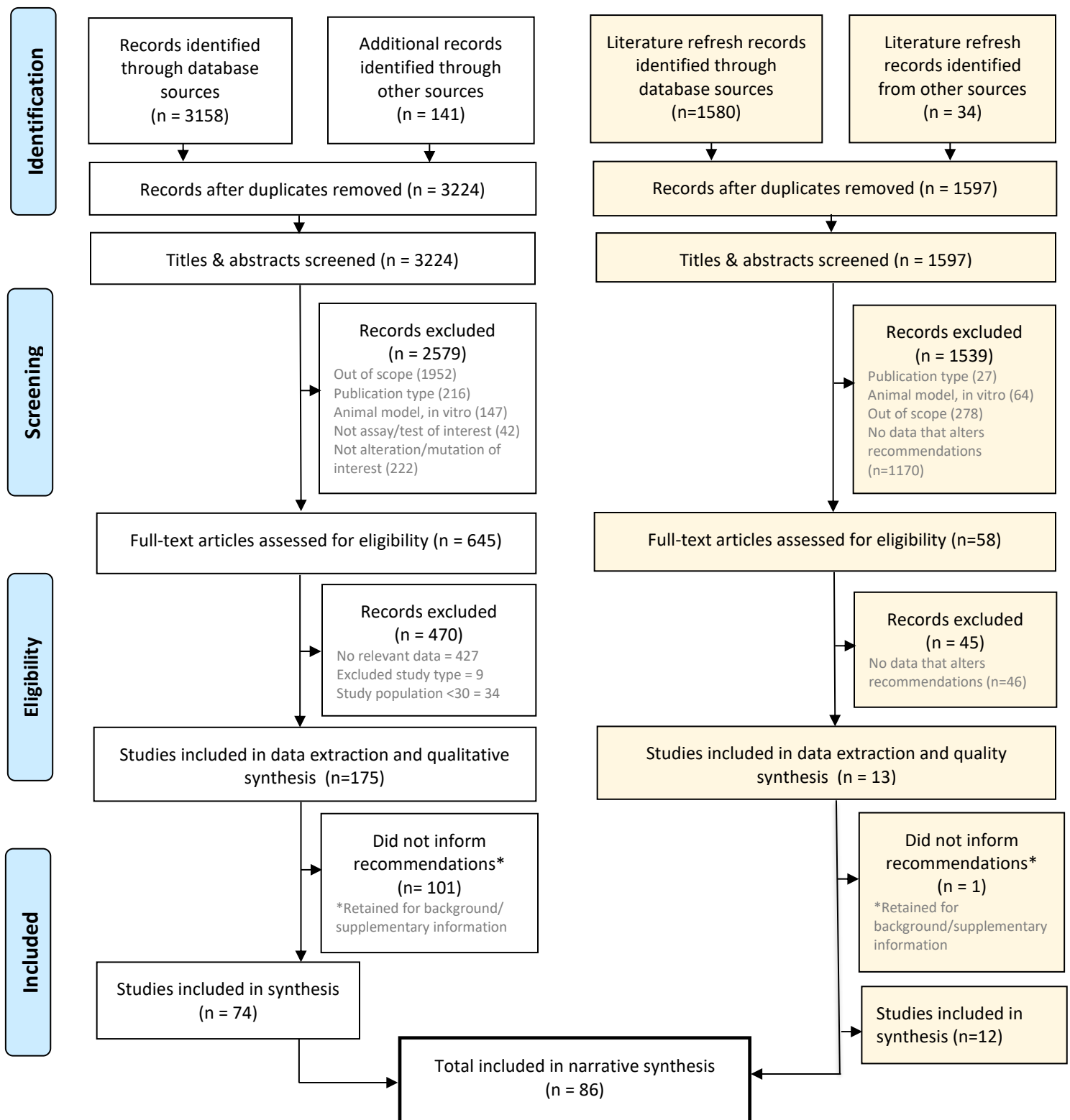
braf* OR atrx* OR p53* OR tp53 OR myb* OR myb?l* OR p?tert* OR tert* OR fgfr* OR egfr* OR pdgfr* OR c?met* OR cdkn2a* OR mgmt* OR pten OR nf?1 OR mdm?2 OR cdk?4) NEAR/3 (mutation* OR mutated OR mutant OR aberration* OR anomaly OR abnormal* OR defect* OR error OR alteration* OR alteration* OR status* OR detect* OR identif*)):ti,ab) OR (((h3* OR k27m* OR histone?3*) NEAR/3 (methylation* OR mutation* OR mutated OR mutant OR alteration* OR status* OR screen* OR detect* OR identif* OR modif*)):ti,ab) OR (1p19q:ti,ab) OR (((chromosome* OR gene* OR germline*) NEAR/3 (mutation* OR mutated OR mutant OR gain* OR loss* OR deletion OR co?deletion)):ti,ab) OR ((microsatellite NEAR/3 (instability OR status)):ti,ab) OR (msi OR mmr OR 'mismatch repair' OR 'isocitrate dehydrogenase' OR 'epidermal growth factor receptor' OR 'fibroblast growth factor receptor' OR 'platelet derived growth factor' OR 'neurofibromatosis 1' OR 'microsatellite instability' OR 'protein mdm2' OR 'cyclin dependent kinase 4' OR 'histone 3':ti,ab))
AND (('sequence analysis'/de OR 'immunohistochemistry'/de OR 'fluorescence in situ hybridization'/de OR 'in situ hybridization'/de OR 'polymerase chain reaction'/de OR 'microarray analysis'/de OR 'gene expression profiling'/de OR 'subtractive hybridization'/de OR 'comparative genomic hybridization'/de OR 'enzyme immunoassay'/de OR 'molecular diagnosis'/de OR 'dna mutational analysis'/de) OR (((sanger OR next?gen* OR rna* OR msp* OR dna* OR 'high throughput' OR exome OR genome OR genomic OR targeted) NEAR/3 sequenc*):ti,ab) OR ('in situ hybridization':ti,ab OR fish:ti,ab) OR (immunohistochem*:ti,ab OR ihc:ti,ab) OR (microarray:ti,ab OR microdissection:ti,ab OR macrodissection:ti,ab OR methylation:ti,ab OR pyro?sequencing:ti,ab) OR ('polymerase chain*':ti,ab OR pcr*:ti,ab OR qpcr*:ti,ab OR rt?pcr:ti,ab OR 'rt msp':ti,ab) OR (rnaseq*:ti,ab) OR (((methylation OR molecular OR rna OR dna OR amplif*) NEAR/3 (extract* OR probe* OR array*)):ti,ab) OR (((gene* OR molecular OR cytogen* OR genomic OR mutation*) NEAR/3 (diagnos* OR analys* OR analyz* OR stratificatio OR assess* OR detect* OR assay* OR platform* OR array* OR amplif* OR hybridization)):ti,ab) OR (((patholog* OR laborator* OR molecular OR histopathol*) NEAR/3 (validat* OR diagnos* OR test* OR method*)):ti,ab) OR ('snp array':ti,ab OR ngs:ti,ab OR arms:ti,ab OR hplc:ti,ab OR dhplc:ti,ab OR lna:ti,ab OR pna:ti,ab OR lcm:ti,ab OR loh:ti,ab OR sscp:ti,ab OR ms?mlpa:ti,ab OR mlpa:ti,ab OR 'mrc holland':ti,ab OR mmr:ti,ab OR r132h:ti,ab OR cgh*:ti,ab OR arraycgh*:ti,ab) OR ('lab* developed':ti,ab) OR (ffpe:ti,ab OR 'formalin fixed':ti,ab OR 'paraffin embedded':ti,ab)) AND [english]/lim AND [2008-2017]/py AND [embase]/lim) NOT ([animals]/lim NOT [humans]/lim) NOT ([conference abstract]/lim OR [editorial]/lim OR [letter]/lim)) NOT [medline]/lim

Targeted Search String (Ovid MEDLINE)
1   exp astrocytoma/ (36680)
2   gliosarcoma/ (674)
3   oligodendroglioma/ (3599)
4   glioma/ (39034)
5   (glioma* or astrocytoma* or glioblastoma* or gliosarcoma* or oligodendroglioma* or oligoastrocytoma* or GBM or
gliomatosis or oligodendroblastoma* or astroglioma*).tw. (93434)
6   ((astrocytic or glial) adj3 (tumor* or tumour* or neoplasm* or malignan* or cancer* or sarcoma*)).tw. (4935)
7   or/1-6 (106868)
8   Proto-oncogene proteins B-raf/ (8717)
9   Isocitrate Dehydrogenase/ (6312)
10   ATRX protein, human.nm. (376)
11   MGMT protein, human.nm. (1346)
12   Genes, p53/ (15306)
13   Genes,myb/ (364)

14   Tumor Suppressor p53 binding protein 1/ (898)
15   Tumor Suppressor Protein p53/ (52314)
16   Receptor, Epidermal Growth Factor/ (40495)
17   Chromosomes, Human, Pair 19/ (3691)
18   Chromosomes, Human, Pair 1/ (7513)
19   Chromosomes, Human, Pair 10/ (3080)
20   Chromosomes, Human, Pair 7/ (5329)
21   Receptor, Fibroblast Growth Factor, Type 1/ (2350)
22   Receptor, Fibroblast Growth Factor, Type 2/ (2016)
23   Receptor, Fibroblast Growth Factor, Type 3/ (1593)
24   Receptor, Fibroblast Growth Factor, Type 4/ (483)
25   exp Receptors, Platelet-Derived Growth Factor/ (6920)
26   Proto-Oncogene Proteins c-met/ (5322)
27   DNA Mismatch Repair/ (2807)
28   Neurofibromatosis 1/ (9735)
29   Genes, Neurofibromatosis 1/ (864)
30   Microsatellite Instability/ (3234)
31   Proto-Oncogene Proteins c-mdm2/ (5883)
32   Cyclin-Dependent Kinase 4/ (3235)
33   PTEN Phosphohydrolase/ (9412)
34   ((IDH* or BRAF* or ATRX* or p53* or TP53* or MYB* or MYB?L* or p?TERT* or TERT* or
FGFR* or EGFR* or PDGFR* or C?met* or CDKN2A* or MGMT* or PTEN* or NF?1* or MDM?2*
or CDK?4*) adj3 (mutation* or mutated* or mutant* or alteration* or aberration* or anomaly*
or anomalies* or abnormal* or defect* or error* or status* or detect* or identif*)).tw.(69011)
35   ((H3* or k27M* or histone?3*) adj3 (mutation* or mutated* or mutant* or methylation*
or alteration* or status* or screen* or detect* or identif* or modif*)).tw. (8241)
36   1p?19q*.tw. (244)
37   ((chromosom* or gene* or germline*) adj3 (mutation* or mutated* or mutant* or
alteration* or aberration* or
anomaly* or anomalies* or abnormal* or defect* or gain* or loss* or deletion* or
co?deletion*)).tw. (322592)
38   (microsatellite adj3 (instability or status)).tw. (7796)
39   (MSI or MMR or "mismatch repair").tw. (19732)
40   or/8-39 (517996)
41   exp Sequence Analysis/ (392408)
42   exp Immunohistochemistry/ (596217)
43   exp In Situ Hybridization/ (93157)
44   exp Polymerase Chain Reaction/ (447902)
45   exp Microarray Analysis/ (92239)
46   exp Gene Expression Profiling/ (131097)
47   Comparative Genomic Hybridization/ (6175)
48   Immunoenzyme Techniques/ (67873)
49   (Immunohistochem$ or IHC).tw. (358820)
50   ((Sanger or next?gen$ or RNA$ or DNA$ or MSP$ or "high throughput" or exome or
genome or genomic or targeted) adj3 sequenc*).tw. (277851)
51   (microarray or microdissection or macrodissection or methylation or pyro?sequencing).tw.
(194617)
52   ("polymerase chain" or PCR* or qPCR* or RT-PCR or RT-MSP).tw. (661933)

53   ("in situ hybridization" or FISH).tw. (237349)

54   (RNAseq$ or ((methylation or molecular or RNA or DNA or amplif*) adj3 (extract* or probe* or array*))).tw. (92932)

55   ((gene* or molecular* or cytogen* or genomic* or mutation*) adj3 (diagnos* or analys* or analyz* or stratification or assess* or detect* or assay* or platform* or array* or amplif* or hybridization)).tw. (593093)

56   "lab* developed".tw. (848)

57   ("SNP array" or NGS or ARMS or HPLC or DHPLC or LNA or PNA or LCM or LOH or SSCP or MS?MLPA or MLPA or MRC-Holland or MMR or R132H or CGH* or arrayCGH*).tw. (245213)

58   ((histopathol* or laborator* or molecular or patholog*) adj3 (diagnos* or test* or method* or validat*)).tw. (208912)

59   (FFPE or "formalin fixed" or "paraffin embedded").tw. (38968)

60   or/41-59 (2979096)

61   7 and 40 and 60 (7169)

62   limit 61 to (english language and yr="2008 -Current") (4864)

63   limit 62 to (case reports or comment or editorial or letter) (414)

64   62 not 63 (4450)

65   exp animals/ not exp humans/ (4720074)

66   64 not 65 (4361)

67   ("cell line$" or "cell culture$" or "in vitro" or mouse or murine or fish or rat or porcine or mice or dog or horse or animal).ti. (1616785)

68   66 not 67 (4208)

69   remove duplicates from 68 (4183)

70   cell line, tumor/ or cells, cultured/ (818907)

71   69 not 70 (3495)

72   limit 71 to yr="2016 -Current" (1712)

73   (pediatric or bithalamic or infantile or infant).tw,kf. (507916)

74   72 and 73 (175)

Supplemental Figure 2: Literature Review Flow Diagram

Identification

Records identified through database sources (n = 3158)

Additional records identified through other sources (n = 141)

Literature refresh records identified through database sources (n=1580)

Literature refresh records identified from other sources (n = 34)

Records after duplicates removed (n = 3224)

Records after duplicates removed (n = 1597)

Screening

Titles & abstracts screened (n = 3224)

Titles & abstracts screened (n = 1597)

Records excluded (n = 2579)
Out of scope (1952)
Publication type (216)
Animal model, in vitro (147)
Not assay/test of interest (42)
Not alteration/mutation of interest (222)

Records excluded (n = 1539)
Publication type (27)
Animal model, in vitro (64)
Out of scope (278)
No data that alters recommendations (n=1170)

Full-text articles assessed for eligibility (n = 645)

Full-text articles assessed for eligibility (n=58)

Eligibility

Records excluded (n = 470)
No relevant data = 427
Excluded study type = 9
Study population <30 = 34

Records excluded (n = 45)
No data that alters recommendations (n=46)

Studies included in data extraction and qualitative synthesis (n=175)

Studies included in data extraction and quality synthesis (n = 13)

Included

Did not inform recommendations* (n= 101)
*Retained for background/supplementary information

Did not inform recommendations* (n = 1)
*Retained for background/supplementary information

Studies included in synthesis (n = 74)

Studies included in synthesis (n=12)

Total included in narrative synthesis (n = 86)

Adapted from Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

Supplemental Figure 3: Targeted Search Review Flow Diagram
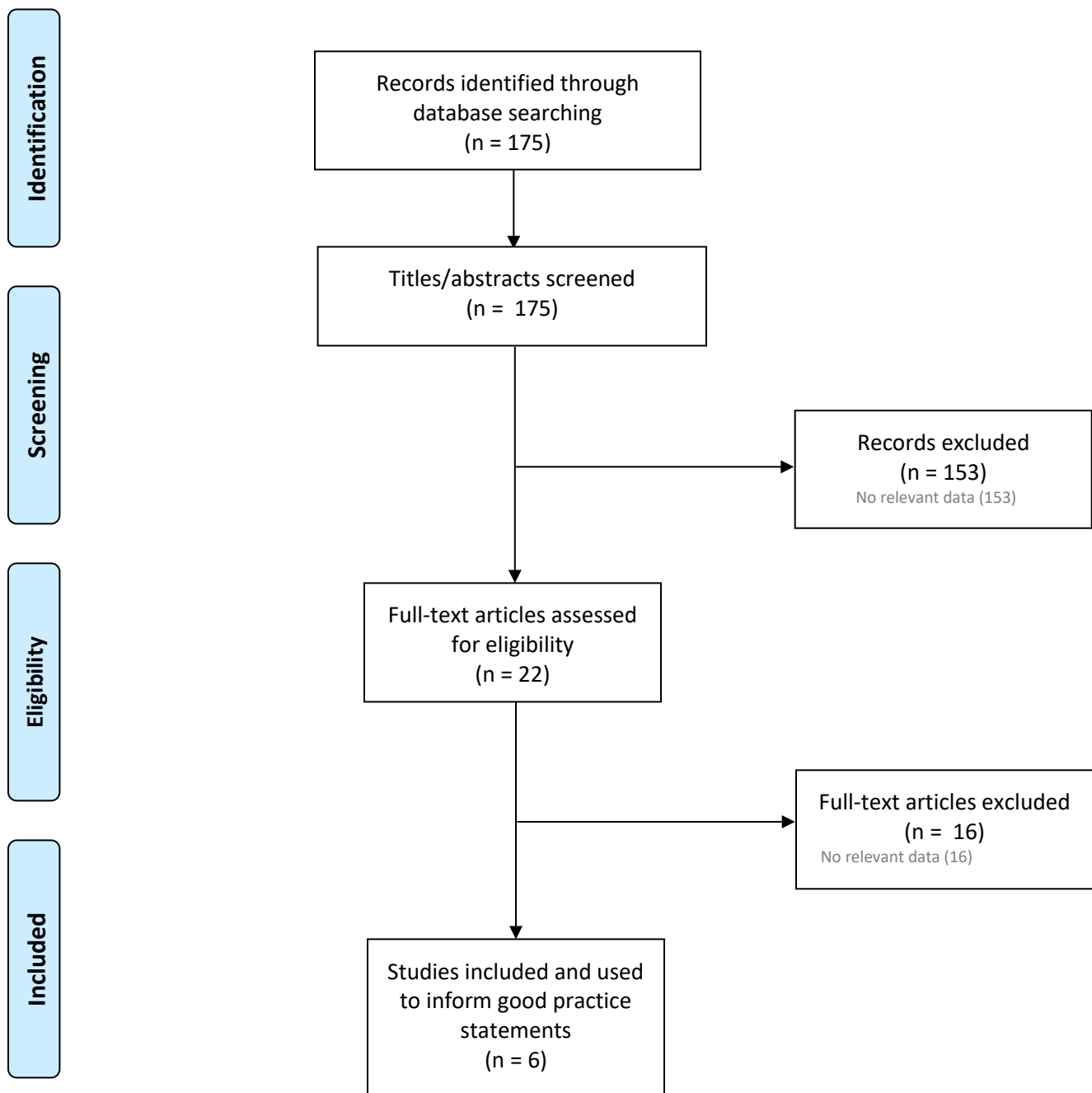
Adapted from Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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