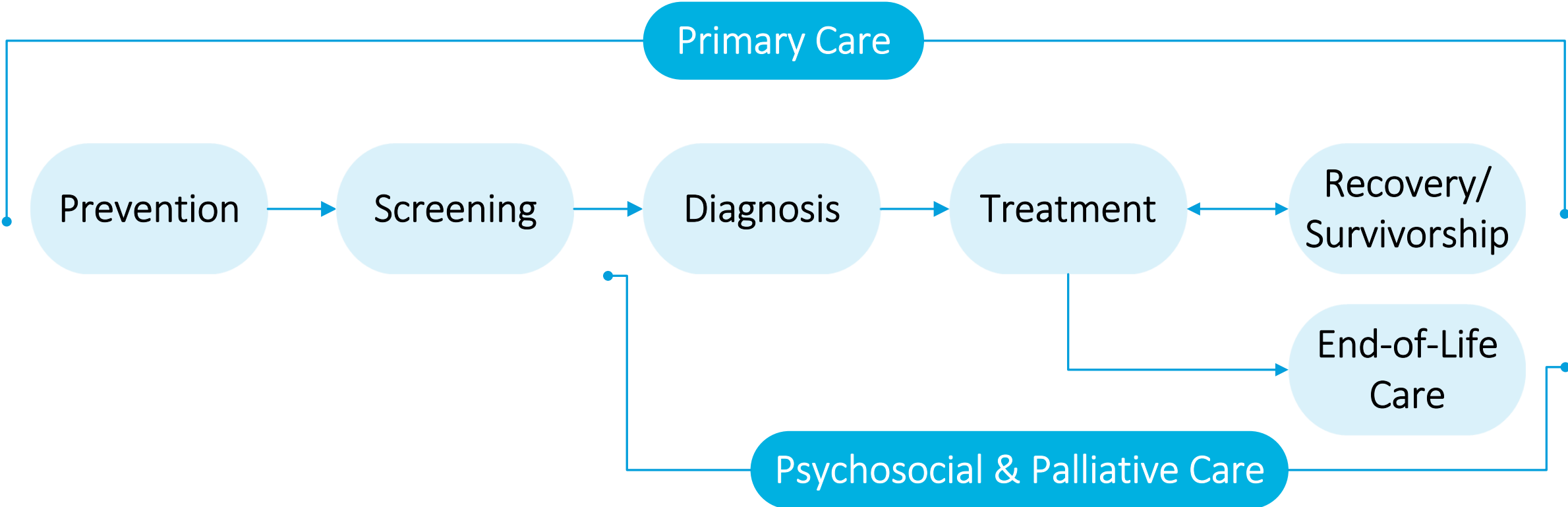


Glioma Reflexive Testing Algorithm

Version 2025.10



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Ontario Health
Cancer Care Ontario

Target Population

CNS cancer patients who present with signs or symptoms suspicious of high or low grade gliomas.

Algorithm Considerations

- MGMT gene promoter methylation testing is recommended for all diffuse gliomas¹
- Recommend a tiered approach with IHC first followed by sequencing if needed (e.g., BRAF fusions)²
- In a patient 55 years of age or older at diagnosis with a histologically classic glioblastoma not located in midline structures and with no history of a pre-existing lower-grade glioma, IDH1 R132H-Negative by immunohistochemistry is sufficient for diagnosis of “Glioblastoma, IDH-wildtype”, without proceeding to *IDH1/2* sequencing³
- MMR IHC and H3G34 IHC is recommended in AYA patients with HGG (see page 4)
- In confirmed cases of MMRD, tumour mutational burden should be performed
- If the diagnosis is unconfirmed after following this molecular workup algorithm and neuropathologist opinion x2, consider genome-wide profiling of CpG methylation patterns⁴

¹Kinslow CJ, Mercurio A, Kumar P, et al. Association of *MGMT* Promotor Methylation With Survival in Low-grade and Anaplastic Gliomas After Alkylating Chemotherapy. *JAMA Oncol*. Published online May 18, 2023. doi:10.1001/jamaoncol.2023.0990

²Lim-Fat MJ, Macdonald M, Lapointe S, et al. Molecular testing for adolescent and young adult central nervous system tumors: A Canadian guideline. *Front Oncol*. 2022 Sep 23;12:960509. doi: 10.3389/fonc.2022.960509. PMID: 36249063; PMCID: PMC9559579.

³Louis DN, Aldape KD, Capper D, et al. Glioblastoma, IDH-wildtype, WHO Classification of Central Nervous System Tumours. 5th Edition. IARC; 2021, page 53.

⁴Capper D, Jones DTW, Sill M, et al. DNA methylation-based classification of central nervous system tumours. *Nature*. 2018 Mar 22;555(7697):469-474. doi: 10.1038/nature26000. Epub 2018 Mar 14. PMID: 29539639; PMCID: PMC6093218.

The following algorithms have been adapted from Lim-Fat MJ, Macdonald M, Lapointe S, et al. Molecular testing for adolescent and young adult central nervous system tumors: A Canadian guideline. *Front Oncol*. 2022 Sep 23;12:960509. doi: 10.3389/fonc.2022.960509. PMID: 36249063; PMCID: PMC9559579.

Algorithm Legend

Colour Guide

Immunohistochemistry

Molecular sequencing

Diagnosis

Shape Guide

Intervention/Test

Decision or assessment point

Patient (disease) characteristics

Consultation with specialist

Diagnosis

or

Off page reference

Line Guide

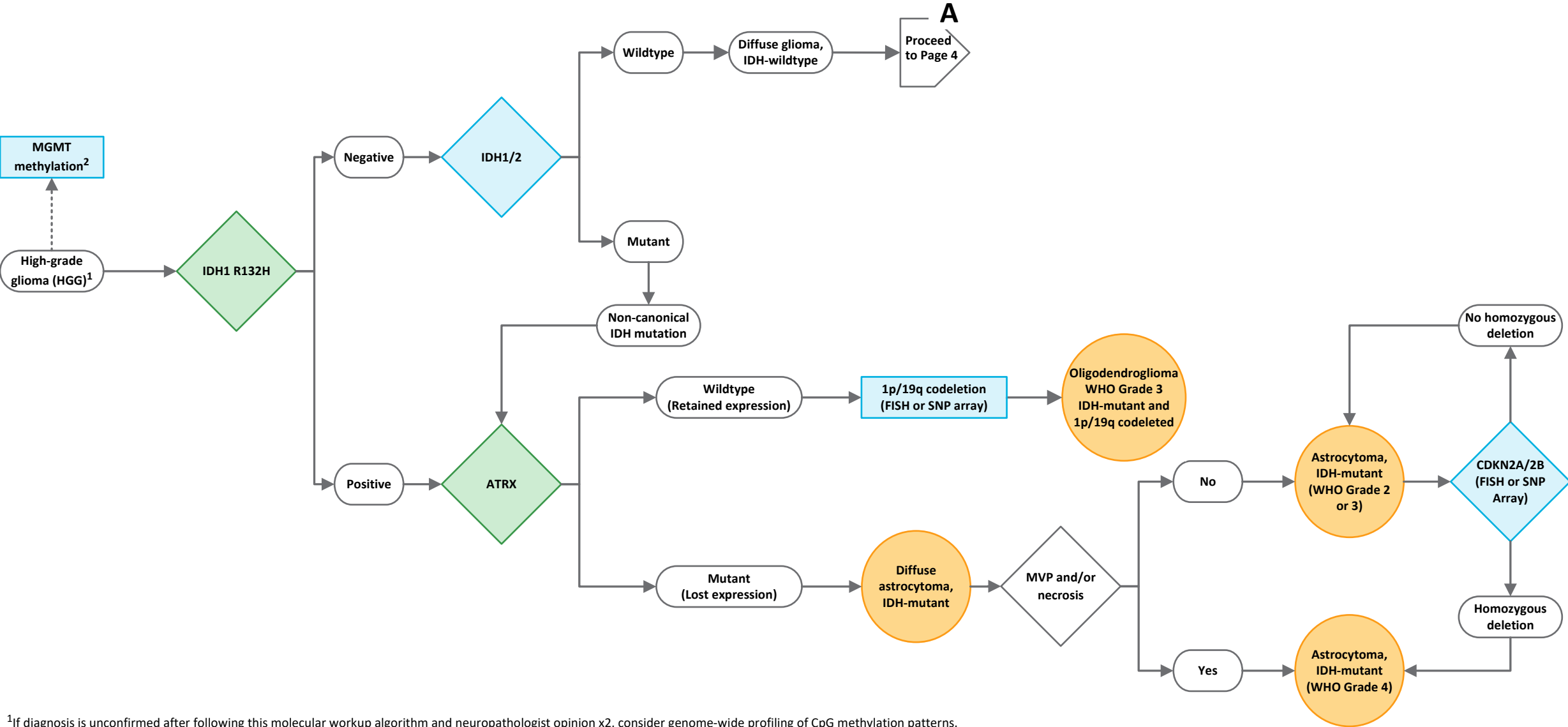
Required

Possible

Abbreviations:

- ATRX – alpha-thalassemia/mental retardation syndrome X-linked
AYA – adolescents and young adults
BRAF – B-Raf
CDKN – cyclin-dependent kinase inhibitor
cMMRD – constitutional mismatch repair deficiency
DMG – diffuse midline glioma
EGFR – epidermal growth factor receptor
EZHIP – EZH Inhibitory Protein
FGFR – fibroblast growth factor receptor
FISH – fluorescence in situ hybridization
HGG – high grade gliomas
IDH – isocitrate dehydrogenase
IHC – immunohistochemistry
LGG – low grade glioma
MAPK – mitogen-activated protein kinase
MGMT – O6-methylguanine-DNA methyl-transferase
MMR – mismatch repair
MMRD – mismatch repair deficiency
MYB – avian myelobalstosis viral oncogene
MYBL1 – MYB Proto-Oncogene Like 1
MYCN – N-myc proto oncogene
NTRK – neurotrophic tyrosine receptor kinase
PXA – pleomorphic xanthoastrocytoma
RNA – ribonucleic acid
RTK – receptor tyrosine kinase
SNP – single nucleotide polymorphism
TERT – telomerase reverse transcriptase
WHO – World Health Organization

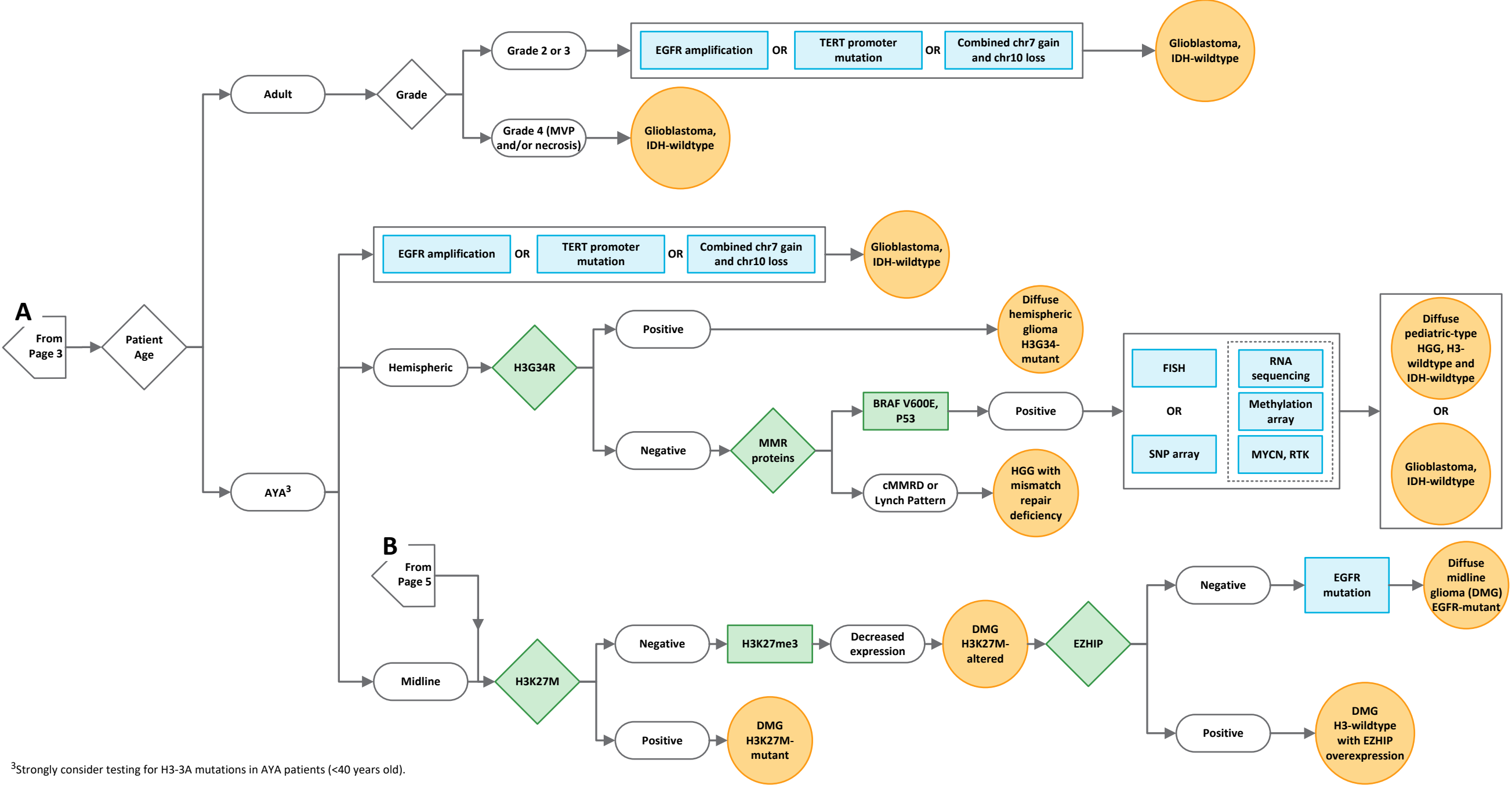
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¹If diagnosis is unconfirmed after following this molecular workup algorithm and neuropathologist opinion x2, consider genome-wide profiling of CpG methylation patterns.

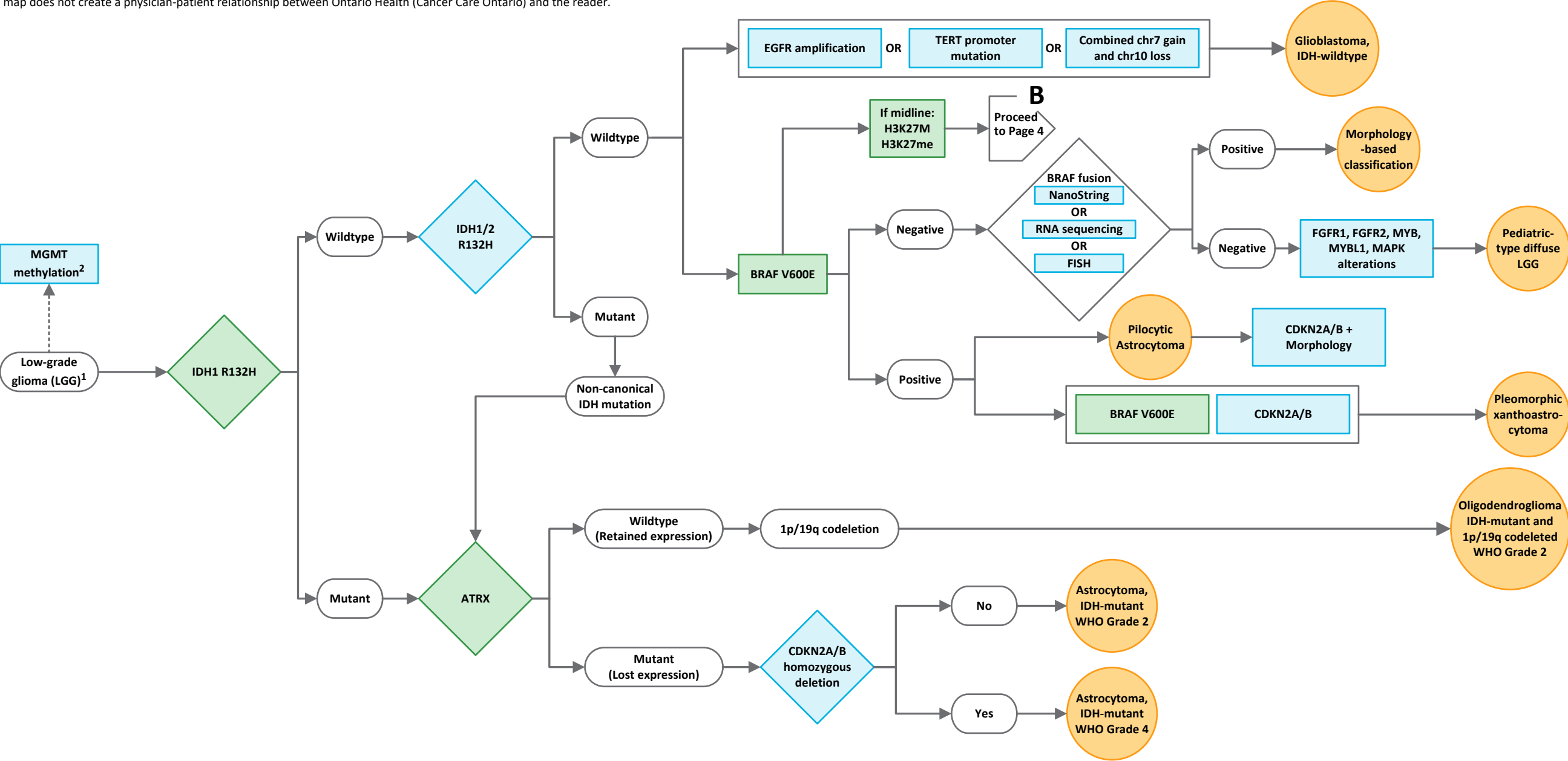
²Testing for MGMT gene promoter methylation is encouraged in all diffuse gliomas.

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³Strongly consider testing for H3-3A mutations in AYA patients (<40 years old).

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