

Golden rules for optimizing the diagnostic pathway of IDH-mutant tumors: bridging evidence and clinical practice in cholangiocarcinoma and adult-type diffuse gliomas

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Summary

Background. Mutations in the isocitrate dehydrogenase (IDH) genes are key biomarkers in intrahepatic cholangiocarcinoma (CCA) and adult-type diffuse gliomas, although real-world adoption of comprehensive molecular diagnostics remains uneven. This review aimed to integrate published evidence, clinical experience, and international guidelines to provide pragmatic recommendations that can standardize IDH testing across healthcare systems.

Methods. A multidisciplinary panel synthesized data identified through 10 PICO-driven questions, critically appraised guideline statements from ESMO, EANO, NCCN, and WHO-CNS5, and incorporated insights from clinical evidence on IDH molecular profiling in CCA patients. Recommendations were developed through interactive expert discussion.

Results. The panel addressed six issues: (i) positioning of next-generation sequencing (NGS) as a first-line assay; (ii) using liquid biopsy to supplement inadequate or uninformative tissue-based molecular analyses; (iii) tumor-adapted workflows combining immunohistochemistry, PCR, or NGS with large genomic panels; (iv) optimizing pre-analytical management of small biopsies in terms of neoplastic cell abundance and nucleic acid fragmentation to safeguard material for integrated testing; (v) evaluating promising biomarkers based on genome-wide methylation profiling and metabolic imaging in specialized centers; and (vi) novel testing strategies including centralized and decentralized algorithms. In addition, emerging approaches based on digital pathology, teleconsultation, and harmonized reimbursement pathways were discussed. These considerations were distilled into a set of "Golden Rules."

Conclusions. Optimized molecular profiling is a cornerstone of precision oncology in IDH-mutant tumors, but the lack of harmonized procedures hinders its widespread implementation in the clinical setting. In intrahepatic CCA, upfront NGS should be prioritized to capture the full spectrum of actionable alterations, whereas in diffuse gliomas IHC for IDH1 p.R132H remains recommended, with PCR or NGS reserved for IHC-negative or equivocal cases. Advanced tools such as genome-wide methylation profiling or metabolic imaging may add value in specialized centers. The consensus-based "Golden Rules" pragmatically support harmonization of diagnostic workflows, reducing technical costs and turnaround time, and promoting equitable access to IDH-directed therapies across diverse healthcare settings.

Keywords: IDH mutations, next-generation sequencing, cholangiocarcinoma, diffuse glioma; glioma, precision oncology

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Introduction

Precision oncology has profoundly transformed the clinical management of solid tumors by stratifying patients eligible for targeted treatments. Isocitrate dehydrogenase 1 and 2 (IDH1/2) actionable mutations have emerged as clinically relevant targets in intrahepatic cholangiocarcinoma (CCA) and adult-type diffuse gliomas, allowing the selection of patients who may benefit from targeted therapy¹⁻⁴.

It has been established that IDH1/2 actionable mutations drive tumorigenesis through the release of the oncometabolite 2-hydroxyglutarate (2-HG) from metabolic pathways⁵. These gain-of-function mutations convert isocitrate to D-2HG rather than α -ketoglutarate, leading to pathological intracellular accumulation. Elevated 2-HG competitively inhibits α -ketoglutarate-dependent dioxygenases, including TET-family DNA hydroxylases and histone demethylases, correlating with histone hypermethylation^{6,7}. As a consequence, the resulting “hypermethylator” signature significantly affects differentiation and promotes oncogenesis⁸⁻¹⁰.

In CCA, IDH1 – and less frequently IDH2 – activating mutations occur in 10-20% of intrahepatic cases, defining a distinct molecular subtype with specific clinical outcomes¹¹. Although the prognostic role of IDH actionable mutations remains unclear, their predictive role in identifying CCA patients who are eligible for targeted drugs has been established and endorsed by international and national societies¹²⁻¹⁴.

Approximately 90% of IDH-mutant gliomas harbor the IDH1 p.R132H mutation, which can be detected using immunohistochemistry with an antibody against the mutant epitope¹⁵. Among the remaining 10%, about 5% exhibit alternative mutations in IDH1 at residue R132, while another 5% show mutations in IDH2 at residue R172. DNA sequencing is necessary to identify IDH2 mutations and IDH1 variants that deviate from p.R132H, referred to as non-canonical IDH mutations^{16, 17}.

Recent clinical trials have validated IDH-activating mutations as therapeutic targets. The phase III Clar-IDHy trial showed that the IDH1 inhibitor ivosidenib significantly improved progression-free survival compared with placebo (median 2.7 vs 1.4 months; HR ~0.37) in patients with CCA¹¹. In addition, the phase III INDIGO trial demonstrated that the dual IDH1/2 inhibitor vorasidenib significantly improved median progression-free survival (27.7 vs 11.1 months) in patients with IDH-mutant glioma, delaying the need for chemo-radiotherapy in those with grade 2 IDH-mutant glioma^{11, 18-21}.

In parallel, the diagnostic landscape has been re-

shaped by technological advances. For CCA, next-generation sequencing (NGS) has become the key platform for simultaneously detecting the wide spectrum of clinically actionable point mutations, copy-number changes, and structural rearrangements²²⁻²⁵. Immunohistochemistry (IHC) remains a rapid and cost-effective method for identifying common IDH1 mutations and selecting patients for targeted inhibitors²⁰. Of note, challenging pre-analytical procedures dramatically affect the testing success rate of tissue specimens, leaving 20-25% of patients who are ineligible for molecular testing²⁶. In this scenario, alternative sources of nucleic acids to complement tissue-based molecular analysis are needed. Liquid biopsy provides a reliable, easily obtainable, and dynamic source of nucleic acids when tissue-based approaches are inadequate. In particular, circulating tumor DNA (ctDNA) has shown high concordance with tissue testing in CCA cohorts where biopsy is infeasible, demonstrating a clinical role in the molecular profiling of IDH mutations²⁷.

In gliomas, advanced imaging and metabolic tools – such as magnetic resonance spectroscopy for 2-HG – are being investigated as non-invasive adjuncts, highlighting how integrative resources may optimize clinical management²⁸. These approaches may also improve clinical algorithms for CCA through comprehensive tumor investigation of both genomic and metabolomic markers. However, the clinical implementation of these strategies remains limited: in Europe, fewer than 10% of tumor samples eligible for molecular testing are profiled using NGS, reflecting disparities between referral centers and peripheral hospitals²⁹. Consequently, several patients are only partially profiled or tested outside the optimal clinical framework, compromising access to mutation-specific therapies. International guidelines consistently underscore the central role of IDH testing. Guidelines from ESMO, NCCN, and AIOM advocate routine IDH testing in advanced CCA disease, including *FGFR2*, *RET* and *NTRK* fusions, *BRAF* mutations, and HER2 overexpression/*ERBB2* amplification^{13, 14}. For diffuse gliomas, the 2021 WHO classification and EANO guidelines identify IDH profiling as a crucial diagnostic procedure, while the NCCN recommends mandatory IDH1/2 testing for prognostic assessment, therapeutic decisions, and clinical trial eligibility, within an integrated molecular framework that also includes markers such as 1p/19q codeletion, ATRX, TP53, and TERT promoter status³⁰⁻³⁵. These recommendations are grounded in strong evidence, including randomized trials and expert consensus. Unfortunately, testing algorithms remain inconsistent, particularly outside referral centers, due to logistical and technical limita-

tions²¹.

Despite harmonized guidelines supporting IDH testing in diagnostic workflows, translation into practice remains fragmented. Advanced diagnostic capabilities – such as highly trained personnel, technical equipment, and resources – are concentrated in tertiary centers, whereas community institutions often face limitations in laboratory infrastructure, workforce, and turnaround times^{29, 36}. Timely and accurate molecular testing is therefore critical not only for disease classification and prognosis but also for enabling targeted treatments²¹.

The rationale behind this project was to bridge the gap between clinical recommendations and real-world practice by supporting the operational application of existing guidelines. We aimed to provide a structured synthesis of available evidence and illustrate how it

can be translated into practical, context-sensitive solutions across diverse healthcare settings. By emphasizing feasibility, equity, and clinical utility, we sought to improve the timely implementation of IDH testing, thereby ensuring broader and more consistent access to molecular diagnostics and targeted therapies.

Methods

This project was designed as a structured two-phase approach, combining a systematic literature review with expert interpretation, to address practical issues in the molecular diagnosis of IDH-mutant solid tumors - specifically CCA and diffuse glioma. We sought to identify challenges and propose feasible, real-world solutions to improve the implementation, timeliness, and clinical

Table 1. Clinical questions addressed by the expert panel according to the PICO (Population, Intervention, Comparator, Outcome) framework, guiding evidence synthesis for the consensus-based “Golden Rules.”

#	Clinical Question (PICO Summary)	P (Population)	I (Intervention)	C (Comparator)	O (Outcome)
1	Is NGS superior to PCR and IHC for molecular diagnosis of CCA?	CCA	NGS	PCR, IHC	Sensitivity, specificity, failure rate, ability to detect rare mutations, clinical impact
2	Can liquid biopsy be used for IDH1/2 detection in advanced CCA?	Advanced CCA	Liquid biopsy	Tissue biopsy	Concordance rate
3	In IDH-negative Glioma by IHC, is NGS superior to single-gene assays?	Glioma with IDH-negative IHC	IHC + NGS	IHC + single-gene assays	Sensitivity, specificity, failure rate, detection of rare mutations, clinical impact
4	Does methylation profiling improve diagnosis in IDH-mutant glioma vs mutation-only analysis?	IDH-mutant glioma	Mutation analysis + methylation profiling	Mutation analysis alone	Prognostic stratification
5	Are multiple biopsies superior to single biopsy for molecular profiling adequacy in CCA?	Advanced CCA	Multiple biopsies	Single biopsy	Failure rate in molecular profiling
6	Does early molecular profiling at CCA diagnosis improve access to targeted therapies?	CCA	Molecular profiling at diagnosis	Molecular profiling at progression	Proportion of patients accessing targeted therapy
7	Does reflex testing increase access to therapy compared to on-demand testing in CCA?	CCA	Reflex testing	On-demand testing	Turnaround time (TAT), access to treatment
8	Does intraoperative histology improve diagnostic adequacy in poor-quality glioma samples?	Glioma with poor-quality surgical specimen	Intraoperative histology + final histology	Final histology alone	Diagnostic adequacy (fewer non-diagnostic biopsies)
9	Can conventional MRI replace biopsy in non-operable glioma for IDH status assessment?	Suspected non-operable glioma	Conventional MRI	Tissue biopsy	Diagnostic accuracy / concordance rate
10	Is MRS a valid alternative for IDH detection in glioma when biopsy is unfeasible?	Glioma not suitable for biopsy	Magnetic Resonance Spectroscopy (MRS) for 2-HG	Biopsy / IHC	Diagnostic concordance

Abbreviations: CCA, cholangiocarcinoma; IDH, isocitrate dehydrogenase; NGS, next-generation sequencing; IHC, immunohistochemistry; PCR, polymerase chain reaction; MLPA, multiplex ligation-dependent probe amplification; MRS, magnetic resonance spectroscopy; FISH, fluorescence in situ hybridization; ctDNA, circulating tumor DNA.

Table II. Operational recommendations (“Golden Rules”) for the implementation of molecular diagnostics in IDH-mutant CCA and gliomas, derived from a synthesis of systematic evidence appraisal and multidisciplinary expert discussion. These recommendations reflect current international guidelines and real-world feasibility across diverse clinical settings.

Setting	Golden Rule	PICO item
CCA	NGS should be implemented as the reference assay at the time of diagnosis.	1
	Liquid biopsy should be considered only in cases where tissue biopsy is contraindicated, unfeasible, or inadequate.	2
	A multi-core biopsy strategy should be adopted to improve adequacy rate of histological evaluation, IHC, and molecular profiling.	5
	Molecular profiling should be performed at diagnosis.	6
	Reflex molecular testing at diagnosis should be preferred over on-demand ordering.	7
Diffuse Glioma	IHC for <i>IDH1</i> p.R132H should be the first-line test, with NGS in negative or equivocal cases.	3
	Genome-wide methylation profiling should be reserved for diagnostically challenging cases in specialized centers.	4
	Frozen section may be selectively used but should not be mandatory for molecular workflows.	8
	Conventional MRI and radiomics should not replace histological confirmation of IDH status due to lack of clinical sensitivity.	9
	2-hydroxyglutarate MRS should be used only in non-biopsiable cases and limited to expert centers.	10

utility of IDH testing in routine diagnostic scenarios.

A multidisciplinary panel of nine Italian experts – including six anatomic pathologists, two molecular biologists, and one medical oncologist involved in the diagnostic or therapeutic management of glioma or biliopancreatic tumor patients – was convened. A first in-person meeting, during which panelists shared institutional experiences and highlighted barriers in clinical practice, was held. Clinically focused questions were then developed using the PICO framework (Population, Intervention, Comparator, Outcome). These covered a broad range of diagnostic issues, from testing techniques to pre-analytical handling procedures and imaging alternatives (Tab. I).

For each PICO question, a dedicated search strategy was built on PubMed. Each search followed a progressive refinement process: an initial broad query was followed by increasingly specific search strings to optimize yield. Search results were limited to articles published within the last 10 years in indexed international peer-reviewed journals. The inclusion criteria were designed to select original studies consistent with the PICO framework – primarily clinical trials (including randomized and prospective studies), real-world cohort analyses, and diagnostic accuracy studies. Reviews, editorials, preclinical investigations, and studies not reporting direct PICO-relevant outcomes were excluded. Titles and abstracts were independently screened by two reviewers; when eligibility was uncertain, full texts were assessed. No quantitative synthesis or meta-analysis was attempted due to heterogeneity in study designs and endpoints.

Results were compiled into structured summaries and circulated among the experts prior to a second-round follow-up online meeting. This meeting served to qual-

itatively interpret the evidence in light of clinical experience, identify potential gaps, and propose practical solutions. No formal consensus methodology (e.g., Delphi rounds or voting) was applied. Instead, the process aimed to synthesize structured insights informed by both published data and real-world expertise, ultimately producing a set of actionable recommendations (“Golden Rules”) to support clinical decision-making (Tab. II).

Results

CHOLANGIOCARCINOMA (CCA)

The most recent international guidelines agree on a two-step framework: universal molecular profiling at treatment decision points and mutation-directed therapy when actionable alterations are detected in CCA patients. The ESMO 2024 Clinical Practice Guideline was updated to recommend upfront comprehensive genomic testing “before or during first-line therapy,” including an NGS panel covering *IDH1* activating mutations as well as *FGFR2*, *RET*, *NTRK*, *BRAF*, and *ERBB2* clinically relevant alterations, in line with the ESMO ESCAT actionability scale. In the same context, the NCCN Biliary Tract Cancers Guideline (Version 2.2025) integrated *IDH1/2* molecular analysis into the panel of mandatory biomarkers for testing. As recommended, broad NGS panels should be adopted to guide targeted therapy selection and clinical trial referral in patients with unresectable or metastatic disease^{13, 14}.

NGS has consistently emerged as the reference method for molecular profiling in CCA patients. Across

nine eligible studies – mostly retrospective cohorts – NGS demonstrated significantly higher technical performance in detecting IDH1/2 mutations, FGFR2 fusions, and rare point mutations (PICO 1), compared with conventional IHC and RT-PCR strategies³⁷⁻⁴⁵. Failure rates were generally low (< 10%) even in small and/or archival samples, and concordance with orthogonal technologies (FISH or dPCR) was consistently moderate to high. Of note, several cases revealed therapeutic targets detectable only by NGS, highlighting its diagnostic superiority over single-gene or stepwise testing. Although technical heterogeneity prevents precise quantification of the diagnostic accuracy of these methodologies, both the literature and international guidelines designate NGS as the reference platform for single-step, comprehensive molecular profiling, driven more by technical sensitivity than by direct outcome-level validation³⁷⁻⁴⁵.

Liquid biopsy represents a promising complementary tool for IDH1/2 detection in advanced CCA (PICO 2). Six studies – including large retrospective series and prospective single-center cohorts – reported high concordance rates between plasma and tissue analysis for IDH1 mutations, reaching 100% in pretreatment samples⁴⁶⁻⁵¹. IDH2 concordance rates were also high in a few cases but remain underexplored. Sensitivity declined when samples were collected during therapy or in stable disease, and no data are available assessing the clinical impact on treatment decisions. Thus, liquid biopsy may be considered in selected or unfit patients, but current evidence does not support its use as a substitute for tissue testing. On this basis, liquid biopsy should be regarded as an integrative source of nucleic acids when tissue-based molecular profiling faces limitations⁴⁶⁻⁵¹.

Pre-analytical management of tissue samples remains affected by challenging and non-harmonized procedures. The adequacy of tissue biopsies is another critical determinant of successful profiling for clinically relevant biomarkers (PICO 5). Although direct CCA-specific evidence is lacking, data from large cohorts (> 1100 biopsies, including pancreatobiliary tumors) show that up to 18.5% of single-core samples are inadequate for molecular testing, whereas multiple-core sampling strategies may represent a valid option in clinical practice (> 80% adequacy). No predictive factors – such as sampling order or lesion site – were identified, suggesting that tissue adequacy primarily depends on the number of cores. Consensus therefore favors a planned biopsy approach to ensure sufficient material for both histological and molecular analyses⁵².

The timing of molecular profiling also emerged as a key issue (PICO 6). Two studies reported that mo-

lecular testing is often performed only after disease progression, thereby excluding many patients from targeted therapies due to poor performance status^{53, 54}. Although actionable alterations were identified in 59% of patients, only a small fraction (30%) received treatment based on their molecular profile, mostly in later lines⁵⁴. This indirectly suggests that profiling at diagnosis – as recommended by guidelines – maximizes therapeutic opportunities. Evidence directly comparing reflex versus on-demand testing is lacking in CCA (PICO 7), leaving an important organizational gap, despite data from other tumor types showing clear advantages of reflex testing strategies.

GLIOMAS

The WHO 2021 CNS classification and EANO 2021 guidelines recommend IDH profiling as an essential diagnostic criterion in adult-type diffuse gliomas: tumors are defined as IDH-mutant (astrocytomas or, if 1p/19q-codeleted, oligodendrogliomas) versus IDH-wildtype (glioblastomas). Current protocols (e.g., EANO and NCCN) consider IDH testing mandatory for all diffuse gliomas. The EANO 2021 guideline on rational molecular testing echoes this mandate, supporting frontline immunohistochemistry for the common IDH1 p.R132H protein, followed by targeted sequencing or multigene NGS analysis to detect non-canonical IDH1/IDH2 variants, assess prognosis, and determine trial eligibility. Consistently, the NCCN Central Nervous System Cancers Guideline (Version 2.2025) lists IDH1/2 mutation assessment – alongside 1p/19q codeletion, ATRX, TP53 and TERT promoter molecular analyses – as mandatory in the diagnostic algorithm for all adult diffuse gliomas, with molecular classification guiding radiotherapy, chemotherapy, and eligibility for emerging mutant-IDH inhibitors³⁰⁻³⁵. NGS is particularly relevant when IHC for IDH1 p.R132H is negative in diffuse glioma patients. Two studies confirmed that NGS outperforms IHC and multiplex ligation-dependent probe amplification (MLPA) in detecting IDH mutations, especially non-canonical variants that are systematically missed by conventional methods (PICO 3). NGS enabled reclassification of misdiagnosed cases, in line with WHO CNS5 criteria, thereby improving eligibility for targeted therapies. Its added value also lies in the simultaneous assessment of additional markers such as 1p/19q codeletion, TP53, ATRX, and TERT promoter mutations^{55, 56}.

Methylation profiling has been explored as a tool to refine diagnosis and prognosis (PICO 4). Several studies have validated methylation classifiers since the seminal work by Capper and colleagues, confirming their diagnostic robustness⁵⁷⁻⁶¹. A multicenter cohort of 122 grade 2/3 gliomas demonstrated that methyl-

ation-based subgroups correlated with survival and resistance patterns, offering additional prognostic information beyond genetics⁶². However, in this specific setting, methylation profiling does not provide a direct confirmation of IDH1/IDH2 mutational status, and a proportion of cases – including some IDH-mutant gliomas – may remain unclassifiable. Current evidence on its prognostic utility in IDH-mutant gliomas is still limited, and its use cannot yet be recommended as part of routine workflows.

Attempts to enhance diagnostic adequacy in suboptimal surgical samples by combining intraoperative histology with final pathology lack supporting evidence (PICO 8). No eligible studies addressed this question, leaving current practice dependent on local expertise. For PICO 9, eight studies were included, consistently showing that conventional MRI features – particularly the T2-FLAIR mismatch sign – achieve excellent specificity (> 97%) but poor sensitivity (approximately 25-50%) for detecting IDH-mutant astrocytomas. Meta-analyses confirmed that, while a positive mismatch sign can reliably indicate mutation, many true IDH-mutant cases are missed. Advanced radiomic and AI-based methods achieved moderate improvements, with diagnostic accuracies of 70-80%, but further investigations are required before they can replace histological analysis⁶³⁻⁷⁰.

Two eligible studies demonstrated that magnetic resonance spectroscopy (MRS)-based detection of 2-hydroxyglutarate provides higher diagnostic accuracy than conventional MRI, with pooled sensitivity around 90% and specificity of 80-85% (PICO 10). A prospective study confirmed similar accuracy using optimized protocols. Nonetheless, performance declines in small tumors with a low neoplastic cell fraction, and reproducibility varies across centers, limiting its routine applicability^{71, 72}.

Discussion

International and national guidelines provide a robust methodological framework for the molecular analysis of intrahepatic CCA and glioma. The most recent ESMO and NCCN guidelines recommend comprehensive genomic profiling at diagnosis or during first-line treatment of CCA patients, evaluating IDH1/2 actionable mutations together with other clinically relevant FGFR2, BRAF, RET, NTRK, and HER2 alterations^{13, 14}. These recommendations are grounded in clinical trials demonstrating the efficacy of mutation-directed therapies, classified in accordance with the ESCAT ranking of molecular targets that prioritize IDH1 inhibitors as a pivotal option^{11, 18}. Moreover, the WHO

CNS5 classification and EANO guidelines outline a stepwise approach starting from immunohistochemistry for the canonical IDH1 p.R132H mutation in glioma patients, leveraging PCR or NGS sequencing in negative or equivocal cases, and integrating other markers such as ATRX, TP53, TERT promoter mutations, and 1p/19q codeletion³⁰⁻³⁵. These standards, already widely disseminated, confirm that IDH testing is indispensable not only for diagnostic classification but also for prognostic stratification and eligibility for clinical trials.

Several limitations emerged from our analysis, which are not related to gaps in the quality of the recommendations themselves but to variability in their implementation across different healthcare systems. Real-world data show that NGS systems are still mainly adopted in high-volume institutions. Across Europe, fewer than 10% of tumor samples requiring molecular analysis are processed by NGS platforms, whereas peripheral institutions, due to logistic delays, limited infrastructure, and lack of skilled personnel, implement diagnostic activities using older-generation techniques. On this basis, a non-negligible percentage of patients are only partially tested or selected for molecular profiling without clinical benefit. The systematic review confirmed that NGS is able to detect a broader spectrum of clinically actionable alterations compared with conventional PCR-based or IHC approaches, with low failure rates even in small or archival samples in CCA patients. Of note, NGS platforms also revealed a moderate-to-high concordance rate with orthogonal techniques, significantly paving the way for upfront testing strategies in clinical scenarios. Nevertheless, the heterogeneous landscape of trials and the lack of standardized approaches still prevent the definition of quantitative performance benchmarks. In glioma, formal evidence regarding the role of comprehensive molecular profiling is more limited but indicates that up to 10% of IDH-mutant tumors carry non-canonical variants that cannot be detected by IHC alone. These findings emphasize that the variability observed in clinical practice is not due to methodological uncertainty but to unequal access and inconsistent adherence to established diagnostic algorithms.

The experience of the expert panel underscores the need for targeted interventions aimed at fostering the widespread adoption of best practices already codified in guidelines. Training programs dedicated to molecular pathologists, combined with educational activities for multidisciplinary teams, are essential to increase awareness of diagnostic procedures. The creation of structured diagnostic networks connecting peripheral hospitals and specialized centers for advanced assays plays a key role in optimizing diagnostic pro-

cedures for IDH testing. Reflex testing strategies, where histological diagnosis automatically triggers molecular profiling, represent a promising approach. Evidence from other settings, such as non-small cell lung cancer, shows that the reflex testing approach reduces turnaround times and increases the proportion of patients who actually receive targeted therapy, and similar benefits can be reasonably anticipated for IDH-mutant tumors. Simplification of diagnostic workflows is critical: integrating multiple cores and on-site adequacy assessment improves the success rate of molecular testing in CCA patients, while intraoperative frozen sections may help ensure diagnostic adequacy in borderline glioma cases.

The heterogeneous landscape of diagnostic scenarios represents an unmet need for equitable access to therapeutic innovations. Retrospective series of CCA patients demonstrate that molecular testing after progression often misses therapeutic opportunities due to low performance status. Early profiling, ideally at diagnosis, can increase the chances of selecting patients for targeted agents or clinical trials⁷³⁻⁷⁷. The risk of under-diagnosis or misclassification persists in glioma patients if institutions fail to apply PCR- or NGS-based testing after IHC-negative results. Non-invasive tools such as MRI radiomics or 2-hydroxyglutarate magnetic resonance spectroscopy show promise but cannot yet replace tissue-based molecular analysis. Ensuring that all patients, regardless of where they are treated, can access the same diagnostic standards is therefore a matter of equity. Regional centralization of complex techniques such as NGS or genome-wide methylation profiling, coupled with telepathology platforms and virtual tumor boards, can bridge the gap between academic and peripheral centers⁷⁸⁻⁸⁰. These infrastructures not only standardize quality but also enable multidisciplinary interpretation of results, which is essential for guiding patient management in rare tumors such as CCA and glioma.

Looking forward, further improvements in diagnostic pathways will require the systematic collection of prospective data and continuous updating of protocols in line with technological and therapeutic advances. For instance, while liquid biopsy for IDH1/2 mutations in CCA shows high concordance with tissue in pre-treatment cohorts, prospective studies are needed to clarify the clinical impact on therapeutic decision-making and outcomes. Genome-wide methylation profiling has proven capable of refining prognostic stratification in glioma patients, but infrastructural barriers limit this application to specialized centers⁸¹⁻⁸⁴. The same applies to advanced imaging modalities and metabolic spectroscopy, which are valuable in selected non-biopsiable patients but cannot yet be recommended for

routine use. Continuous updating of diagnostic algorithms is therefore crucial, and prospective audits that monitor the timing, yield, and clinical impact of molecular testing will be essential to ensure progress.

In conclusion, evidence indicates that the real challenge lies in ensuring the consistent and equitable implementation of established diagnostic standards. International and national guidelines already provide clear methodological references; reducing variability, expanding access, and harmonizing diagnostic pathways require education, networking, simplification of workflows, and prospective monitoring – with the ultimate goal of guaranteeing that every patient with IDH-mutant CCA or glioma can benefit from timely and accurate molecular analysis and access to biomarker-driven therapies.

Golden rules

Across both pathologies, the following recommendations reinforce the central role of international and national guidelines as the methodological foundation for molecular diagnostics. The priority now lies not in redefining diagnostic strategies but in ensuring their consistent implementation. Wider dissemination of existing recommendations requires targeted training initiatives and the development of pragmatic operational tools that can be adapted to diverse clinical settings, including peripheral centers. Collaborative platforms, such as regional molecular tumor boards and telepathology infrastructures, can further facilitate adoption in institutions lacking ultra-specialized expertise. Ensuring uniform diagnostic pathways is a prerequisite for equitable access to targeted therapies, and the ongoing collection of prospective data, combined with continuous updating of protocols in line with technological advances, will be essential to sustain progress.

CCA

- In CCA, NGS should be implemented as the reference assay at the time of diagnosis. Evidence from nine eligible studies (mostly retrospective) highlights that NGS is comparable and often superior, in terms of detection rate of IDH mutations, to conventional PCR-based or IHC approaches for detecting clinically actionable alterations, such as IDH1/2 mutations and FGFR2 aberrant rearrangements. Technical failure rates are generally below 10% even on archival material, and concordance with orthogonal techniques such as FISH or PCR ranges from moderate to high. Although outcome-level data are limited, the analytic superiority of NGS and the alignment with ESMO and NCCN

recommendations support its technical implementation in diagnostic settings (PICO 1).

- Liquid biopsy represents a promising, complementary tool for tissue-based molecular analysis. The concordance rate with IDH1 tissue genotyping reached 100% in pretreatment plasma, whereas sensitivity decreased when samples were collected during therapy or in stable disease. Data on IDH2 remain controversial, as no impact on clinical outcomes has been demonstrated. Liquid biopsy should therefore be considered a key tool when tissue biopsy is contraindicated or yields insufficient material (PICO 2).
- Ensuring adequate tissue sampling is critical. Data from 1,100 biopsy cores in oncology patients, including 79 pancreatobiliary tumors, showed an inadequacy rate of up to 18% of cases, while obtaining multiple image-guided cores increases the likelihood of diagnostic adequacy to over 80%. No study has directly compared single versus multiple sampling in CCA, but expert consensus favors a multi-core strategy combined with careful pre-analytical planning and distribution of tissue across histology, IHC, and NGS (PICO 5).
- Molecular profiling should be performed at the time of diagnosis. Observational cohorts demonstrate that more than half of patients harbor actionable alterations, but only a small fraction (30%) receive targeted therapy due to poor performance status or treatment delays. Early testing maximizes the probability of maintaining therapeutic eligibility and should be systematically adopted (PICO 6).
- Reflex molecular testing at diagnosis should be preferred to on-demand ordering. While no study has compared these strategies in CCA, other evidence shows that reflex testing reduces turnaround time, increases testing rates, and improves access to matched therapies. In a disease as aggressive as CCA, reflex NGS at diagnosis is operationally and clinically justified, even in the absence of tumor-specific randomized evidence (PICO 7).

GLIOMA

- In gliomas, the recommended first step remains IHC for IDH1 p.R132H, given its rapidity and cost-effectiveness. After IHC-negative or equivocal results, direct sequencing methods or NGS systems should be performed to identify less common IDH1 variants and IDH2 mutations. This strategy ensures accurate classification under WHO CNS5 criteria and provides simultaneous assessment of additional markers such as ATRX, TP53, 1p/19q codeletion, and TERT promoter mutations. Broad upfront NGS is not recommended, but in IHC-neg-

ative or ambiguous cases it is essential to classify glioma patients in accordance with molecular assessment (PICO 3).

- Genome-wide methylation profiling should not be integrated into routine diagnostic practice. A single-center experience built on 122 tumors demonstrated a correlation between methylation-defined subgroups and clinical outcome, but the design was exploratory and not integrated into routine decision-making. Additional studies have focused only on specific loci rather than comprehensive classifiers. Technical and infrastructural barriers also limit feasibility. As a consequence, methylation profiling should be reserved for selected ambiguous or diagnostically challenging cases in referral centers (PICO 4).
- Intraoperative frozen section analysis may confirm the presence of lesional tissue, but does not reliably improve downstream molecular adequacy. No published studies directly address this question, and its application should remain discretionary, depending on lesion size, trajectory through eloquent cortex, or risk of inadequate sampling. Frozen section should not be mandated for molecular workflows (PICO 8).
- Conventional MRI and advanced radiomic approaches cannot substitute for histological analysis of IDH status. The T2-FLAIR mismatch sign has specificity close to 100% but sensitivity as low as 25-50%, while radiomic and machine-learning models achieve 70-80% accuracy with inconsistent external validation. These methods may inform diagnostic probability but cannot replace tissue-based molecular testing, which remains the gold standard (PICO 9).
- MRS for 2-hydroxyglutarate may be considered in patients with suspected glioma who cannot undergo biopsy due to clinical or anatomical constraints. Meta-analytic data report pooled sensitivity of 93% and specificity of 84%, and a prospective single-center study confirmed 79% sensitivity and 100% specificity with optimized protocols. However, diagnostic performance declines in small or low-cellularity lesions, and reproducibility across centers is limited. MRS should therefore be restricted to expert centers and reserved exclusively for non-biopsiable cases, serving as a supportive adjunct rather than a substitute for histological confirmation (PICO 10).

Conclusions

Mutations in the IDH1 and IDH2 genes have emerged

as pivotal biomarkers in the diagnostic and therapeutic landscape of solid tumors, particularly intrahepatic CCA and diffuse glioma. Molecular analysis not only enables precise disease classification and prognostication but also paves the way for targeted therapies with the potential to improve clinical outcomes.

Despite their established clinical relevance, the implementation of IDH testing within broader integrated molecular diagnostic workflows remains inconsistent across different settings, limited by varying diagnostic pathways, access to advanced technologies, and lack of harmonized protocols. This expert initiative addressed these challenges by combining a systematic review of the evidence with multidisciplinary consensus-building, ultimately delivering contextualized recommendations for the optimal integration of IDH diagnostics into routine clinical practice.

The resulting operational guidelines – organized as “Golden Rules” – support a structured, efficient, and equitable approach to molecular diagnostics in IDH-mutant tumors. Key priorities include early testing at diagnosis, adoption of stepwise or NGS-based workflows, integration with international guidelines, and strategic use of centralized or regional resources to ensure access.

Looking ahead, the continued evolution of IDH-targeted therapies and molecular classification systems will further amplify the need for standardized and sustainable diagnostic strategies. By aligning evidence with real-world feasibility, the recommendations provided here aim to facilitate the implementation of precision oncology for IDH-mutant tumors across diverse clinical settings.

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AUTHOR CONTRIBUTION

M.A., V.B., L.B., B.D., M.F., U.M., N.N., G.P., and A.S. contributed to the conception and design of the project, literature review, and correction of the initial manuscript draft. All authors reviewed and approved the final version of the manuscript.

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