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SAMPLE INFORMATION			
Name	-	**Date Sp. Extracted**	-
ID/Medical ID	-	**Req. Physician**	-
Date of Birth	-	**Report No**	26xxxxxxGR
Material #1	PARAFFIN EMBEDDED TISSUE-BLOCK	**Date Received**	-
Material #2	-	**Date of Report**	-
Sample #1 ID	-	**Tumor type**	CHOLANGIOCARCINOMA

Com.Pl.i.t. Dx (14 genes, 7 fusions) | Comprehensive Panel for Individualized Treatment

1. Clinically Significant Biomarkers*

Biomarker	Result	Therapies with strong clinical significance		Therapies with potential significance	Therapies with potential resistance
		Approved/ Standard of care therapies (Level A)	Therapies in well powered studies (Level B)	Off label/ Investigational therapies (Level C)	
IDH1	Exon 4 p.R132C VAF: 8.4%	Ivosidenib	-	Vorasidenib	-
Microsatellite Instability (MSI)	Stable (MSS)	-	-	-	-

*Note: Variants' Level of Evidence (LoE) (e.g. A, B, C etc) are based on the Joint consensus recommendation of AMP, ACMG, ASCO and CAP for reporting genetic variants in cancer. For a detailed description of the recommendation please refer to Fig. 1

^ The analysis of selected biomarkers included in the present multigene panel is performed within the scope of accreditation according to ELOT EN ISO 15189:2022 (see «Methodology» section). Accreditation according to ELOT EN ISO 15189:2022 applies exclusively to the analytical determination of the selected biomarkers. Interpretations related to the prediction of therapeutic response are not included in the scope of accreditation.

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-

George Nasioulas, PhD Molecular Biologist, Scientific Director, Medical ID:26025301255

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2. Methodology

NGS analysis

DNA is extracted from the sample under investigation using the Qiamp DNA FFPE Kit (Qiagen). A capture based targeted next generation sequencing (NGS) analysis was performed, using the Oncology 188-Gene Variant Assay (GenePlus) which is a qualitative test which enables the simultaneous detection of single nucleotide variants (SNVs), insertions and deletions (InDels), fusions and copy number variations (CNVs) as well as microsatellite instability (MSI) in a single workflow.

Sequencing was carried out on the DNBSEQ-T7 NGS platform (MGI). The analysis of 14 genes includes the entire exon regions and CNVs of 5 genes, partial exon regions of 9 genes and introns/promoters/fusion breakpoint regions of 7 genes. The test also reports on Microsatellite Instability (MSI) status.

Sequencing data are analyzed through bioinformatics pipeline for variant calling and interpretation using the Gene+Box data analysis and management system. Alteration- and tumor type-specific therapeutic implications are classified using the OncoKB™ Levels of Evidence system, which assigns clinical actionability to individual mutational events. For additional details about the OncoKB™ curation process, please refer to the version-controlled OncoKB™ Curation Standard Operating Procedure.

Genes Analyzed

14 Gene Alterations									
BRAF*	CDKN2A*	CTNNB1	ERBB2*	IDH1	IDH2	KRAS	MET	NF1	PTEN*
TERT	TSC1	TSC2	TP53*						
7 Fusion Genes									
FGFR1	FGFR2	FGFR3	NTRK1	NTRK2	NTRK3	RET			
MSI									

*CNV (amplification/deletion) analysis is included for these genes

Biomarkers within the scope of accreditation (ISO15189:2022)

-MSI (NGS)

Sensitivity: Positive reference standards are tested with the assay, all corresponding mutation sites can be accurately detected, and the positive percent agreement (PPA) for all variants (SNVs, Indels, fusions and CNVs) assessed was 95%.
Specificity: Negative reference standards are tested with the assay, and the negative percent agreement (NPA) of SNVs, Indels, fusions and CNVs was 99%.

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Limit of Detection (LoD): The detection limit of the method is 1-2% of mutant allelic content, depending on the genomic region. DNA variations detected at frequencies of less than 5% are confirmed using NGS or an alternative method (Real-Time PCR). All fusions detected are confirmed with an alternative method (Real-Time PCR or FISH).

The performance characteristics of the assay are summarized in the following table:

Variant Type	Limit of Detection
Single nucleotide variations (SNV)	VAF ≥2-5%
Insertions/deletions (Indel)	VAF ≥2-5%
Fusion (or rearrangement)	VAF ≥2%
Copy number (CNV)	≥3 copies

Disclaimer

1. This test is mainly used to assist clinical decision-making, and the result does not represent clinical decision.
2. The test should be interpreted by combining the actual patient context. The medication information provided only on the basis of genetic test results, and the actual medication should follow the physician's instructions.
3. The clinical trials only present partial relevant clinical recruitment trials. For more comprehensive and updated information, please refer to the website: https://clinicaltrials.gov/.
4. As evidence on variants and drugs evolves, previous classifications may later be modified. The interpretation of a variant is based on current available evidence.
5. Sequence variants were reported using Human Genome Variation Society (HGVS) nomenclature. Classification and interpretation of variants follow guidelines of American College of Medical Genetics and Genomics (ACMG), Association of Molecular Pathology (AMP), American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP).
6. Database and references used: Reference genome (GRCh37), annotation using A Locus Reference Genomic (LRG), database referencing 1000G (phaseIII-ucsc), EXAC (0.3.1), dbSNP (147), PolyPhen2/SIFT (ensdb v73), PhyloP (2013-12-06), Clinvar (2018-8) and Cosmic(V80).

Limitations

1. The test is limited to test genomic variations on DNA level and does not involve RNA level or protein level.
2. Limited tissue detection may not represent the whole DNA variations of lesions because of tumor heterogeneity.
3. Scientific data show that not all patients carry genomic variations that are associated with targeted drug, therefore not all subjects can be matched with targeted therapies or clear resistance mechanism.
4. Genetic variation beyond the detection range of this test or some non-gene mutation related factors such as drug interactions may affect the clinical effects of drugs.
5. The detection method cannot distinguish between somatic mutations and germline mutations effectively without control sample analysis.

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6. Every molecular test has an internal 0.5-1% chance of failure. This is due to rare molecular events and factors related to the preparation and analysis of the samples.
7. Improper sample collection, transportation and storage may lead to compromised or invalid results.

3. Quality Control Results

Quality Control Index	Result	Criterion
Average effective sequencing depth[1]	**5239**	≥ 500
Tumor cell content[2]	30%	≥20%
Overall Assessment[3]	PASS	

Note

1. Average effective sequencing depth: Average sequencing depth on target with duplicated reads.
2. An overall tumor cell content percentage of ≥ 20% is recommended for the efficient detection of somatic alterations in the sample analyzed.
3. Overall Assessment: The quality control overall assessment results are divided into two levels: "PASS" and "RISK". When the overall quality assessment result is "RISK", 94-96% of coverage was achieved in the genes analysed, hence there is a small range where clinical actionable variations could be undetected.

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4. Important biomarkers findings

Gene	Finding (VAF)	Gene	Finding (VAF)
*BRAF*_V600E	Not Detected	*NTRK1/2/3* rearrangement	Not Detected
ERBB2 amplification	Not Detected	*MSI* status	**MSS**
FGFR2 rearrangement	Not Detected	*RET* rearrangement	Not Detected
IDH1 mutation	**p.R132C (8.4%)**		

5. Immune Checkpoint inhibitors biomarkers

Biomarker/Variant	Result	Clinical Interpretation
Treatment effect - positive correlation		
MSI status	MSS	-
TP53 mutation	Not Detected	-
Treatment effect - negative correlation		
ALK rearrangement	Not Detected	-
PTEN inactivating mutation	Not Detected	-

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6. Interpretations for targeted therapies

Genetic Variation:	*IDH1*: c.394C>T (p.R132C)	VAF*: 8.4%	OncoKB

Treatment Information

On August 25, 2021, the FDA approved ivosidenib for the treatment of adult patients with unresectable locally advanced or metastatic hepatocellular isocitrate-dehydrogenase-1 (IDH1) mutated cholangiocarcinoma (CCA) as detected by an FDA-approved test with disease progression after 1–2 prior lines of systemic therapy for advanced disease. The approval was based on data from Study AG120-C-005 (ClarIDHy), a double-blind placebo-controlled trial which randomly allocated (2:1) patients to receive either ivosidenib or placebo. Independently-assessed progression free survival (PFS) was the primary endpoint. With a median follow up of 6.9 months, the hazard ratio for PFS was 0.37 (95% confidence interval 0.25, 0.54, p< 0.0001). Overall survival (OS) was the key secondary endpoint. At the final analysis of OS, with 70.5% patients in the placebo arm receiving ivosidenib post disease progression, a non-statistically significant improvement in the ivosidenib arm with a HR = 0.79 (95% CI: 0.56, 1.12) and median OS of 10.3 months (95% CI 7.8, 12.4) and 7.5 months (95% CI 4.8, 11.1) in the ivosidenib and placebo arms respectively were reported. Adverse reactions occurring in >20% of patients receiving ivosidenib were fatigue/asthenia, nausea, diarrhea, abdominal pain, ascites, vomiting, cough, and decreased appetite. Adverse reactions occurring in >20% of patients receiving placebo were fatigue/asthenia, nausea, abdominal pain, and vomiting. This is the first approval for the subset of patients with CCA harboring an IDH1 mutation.

Gene information

The IDH1 (isocitrate dehydrogenase 1) protein is an enzyme that catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG), a crucial step in the tricarboxylic acid (TCA) cycle. IDH1 utilizes NADP(+) as an electron acceptor and it is predominantly expressed in the cytosol and peroxisomes, playing a role in the cytoplasmic production of NADPH. Cancer-associated mutations in the catalytic site of IDH1 confer a gain-of-function of neomorphic enzymatic activity, allowing the mutant enzyme to convert α-KG to the “oncometabolite” D-2-hydroxyglutarate (2-HG) (PMID: 20171147). 2-HG promotes tumor development by inhibiting a variety of enzymes that require α-KG as a substrate, including enzymes involved in DNA demethylation, histone demethylation, adaptation to hypoxia and collagen maturation. IDH1 mutations have been identified in glioma, cholangiocarcinoma and acute myeloid leukemia (AML) among others (PMID: 19657110, 23630074) and may contribute to the progression of myeloproliferative neoplasms to bone marrow failure or AML (PMID: 29355841).

Variant Information

The IDH1 R132C mutation is located in the IDH1 catalytic site. This mutation has been found in acute myeloid leukemia and glioma, among others (PMID: 19935646, 23954893). In vitro and in vivo studies have demonstrated that this mutation changes IDH1 enzymatic activity, allowing for the conversion of α-ketoglutarate (α-KG) to the "oncometabolite" D-2-hydroxyglutarate (2-HG), and leads to oncogenic activity in cooperation with HOXA9, as shown by the ability to induce a myeloproliferative disease-like phenotype and increase downstream signaling and cell cycle progression compared to wildtype IDH1 (PMID: 19935646, 23954893). IDH1 mutations at the R132 residue, including R132C, are sensitive to inhibition by AG-120 (Ivosidenib), a small-molecule inhibitor of mutant IDH1, as measured by a decrease in 2-HG level, an increase in the proportion of mature myeloid cells and increased levels of cell surface differentiation markers in treated cells compared to untreated cells (PMID: 29670690). A Phase I clinical trial for ivosidenib involving 268

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patients with relapsed or refractory AML harboring IDH1 R132 mutations, including 156 with IDH1 R132C mutation, achieved an overall response rate of 41.6% and a reduction in the percentage of bone marrow blasts in treated patients (PMID: 29860938). The IDH1 R132C mutation is known to be oncogenic.

Ivosidenib

DrugBank

Ivosidenib is a small molecule inhibitor of mutant IDH1 that is FDA-approved for the treatment of IDH1-mutant cholangiocarcinoma as detected by an FDA-approved test. FDA approval was based on the results of the Phase III ClarIDHy trial of ivosidenib versus placebo in 185 patients with IDH1-mutant cholangiocarcinoma in which the median progression-free survival was 2.7 months in the patients treated with ivosidenib compared to 1.4 months in the patients receiving placebo, with six- and twelve-month progression-free survival rates in the ivosidenib group of 32% and 21.9%, respectively (HR= 0.37; 95% CI= 0.25-0.54; p < 0.001) (PMID: 32416072). Overall survival was 10.8 months in the ivosidenib group versus six months (adjusted for crossover) for the placebo group (HR= 0.46; p = 0.0008) (PMID: 32416072). Final results demonstrated a mean overall survival of 10.3 months (95% CI= 7.8-12.4 months) with ivosidenib vs 7.5 months (95% CI= 4.8-11.1 months) with placebo (HR= 0.79 [95% CI, 0.56-1.12]; p = .09) (PMID: 34554208).

Vorasidenib

DrugBank

Vorasidenib is an orally available, small molecule inhibitor of IDH1/2 that is FDA approved for the treatment of adult and pediatric patients twelve years and older with grade 2 astrocytoma or oligodendroglioma with a susceptible IDH1 or IDH2 mutation, following surgery including biopsy, sub-total resection, or gross total resection. FDA approval is based on the results of the Phase III INDIGO (NCT04164901) trial of vorasidenib versus placebo in 331 patients with IDH1/2-mutant low-grade glioma. In the Phase III INDIGO (NCT04164901) trial, the vorasidenib cohort (n=168 [n=163, IDH1 mutant; n=5, IDH2 mutant]) demonstrated a median imaging-based progression-free survival (PFS) of 27.7 months (95% CI=17.0-NE) and the time to next intervention (TTNT) was 85.6% (95% CI=77.8-90.8) at eighteen months and 83.4% (95% CI=74.0-89.6) at 24 months while the placebo cohort (n=163 [n=152, IDH1 mutant; n=11, IDH2 mutant]) demonstrated a median imaging-based PFS of 11.1 months (95% CI=11.0-13.7) (HR=0.39 [95% CI=0.27-0.56]; p<0.001) and the TTNT was 47.4% (95% CI=35.8-58.2) at eighteen months and 27.0% (95% CI=7.9-50.8) at 24 months (PMID: 37272516).

*VAF: Variant Allele Frequency

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7. Clinical Trials to consider

IDH1 associated clinical trials

NCT05417594	PHASE1 | PHASE2
Title	Study of AZD9574 as Monotherapy and in Combination With Anti-cancer Agents in Participants With Advanced Solid Malignancies
Treatment	AZD9574 | Temozolomide (TMZ) | [11C]AZ1419 3391 | Trastuzumab Deruxtecan (T-DXd) | Datopotamab Deruxtecan (Dato-DXd)
Location	Australia, Germany, South Korea, Spain, Sweden, United Kingdom, United States

NCT06501625	PHASE1 | PHASE2
Title	Ivosidenib Plus Durvalumab and Gemcitabine/Cisplatin as First-Line Therapy in Participants With Locally Advanced or Metastatic Cholangiocarcinoma With an IDH1 Mutation
Treatment	Ivosidenib | Durvalumab (for the first 8, 21-day, cycles) | Gemcitabine (for the first 8, 21-day, cycles) | Cisplatin (for the first 8, 21-day, cycles) | Durvalumab (starting from cycle 9) | Ivosidenib Recommended Combination Dose (RCD)
Location	Australia, Brazil, Canada, France, Germany, Japan, South Korea, Spain, United States

Press here for a live search of clinical trials for IDH1

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8. Appendix

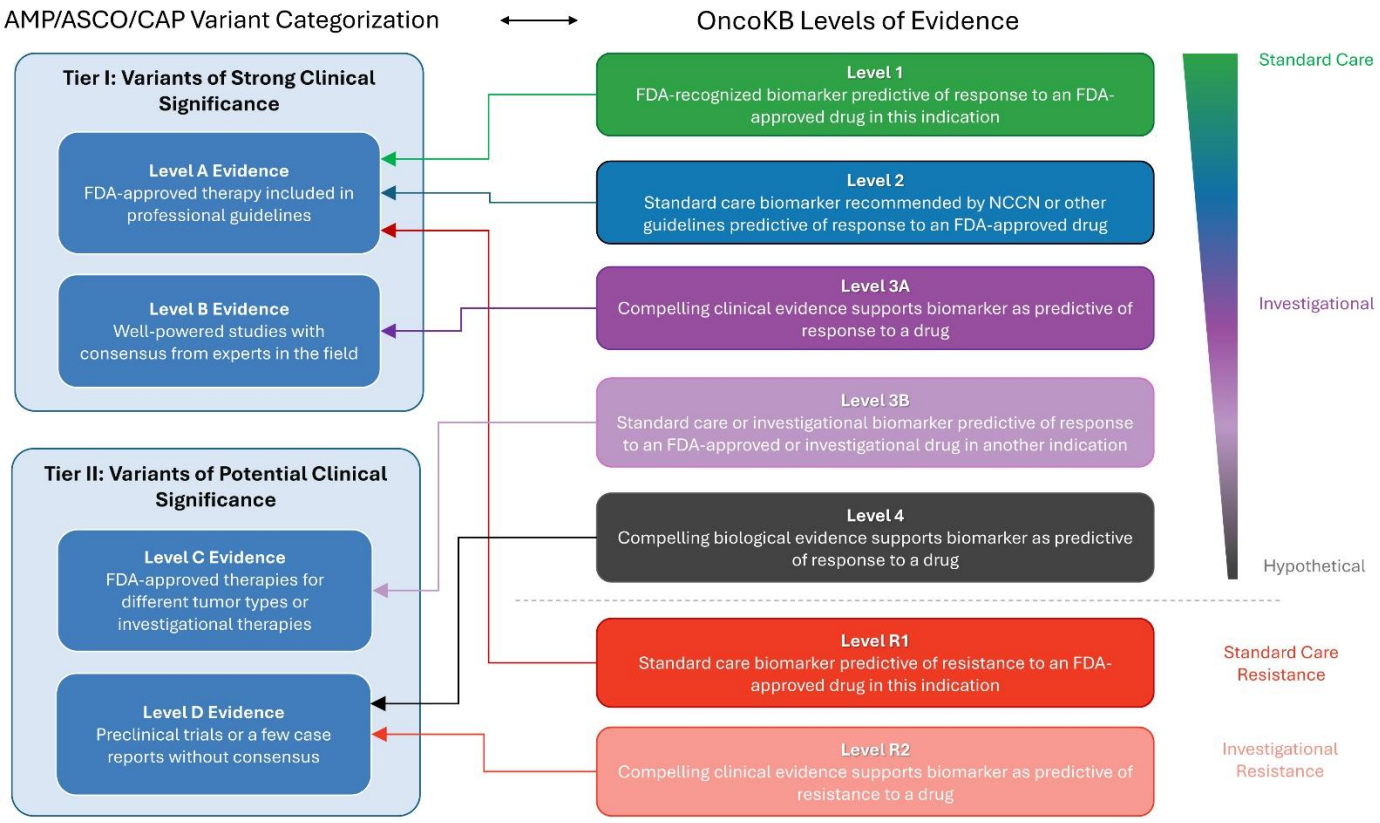

Figure 1. Joint consensus recommendation of AMP, ACMG, ASCO and CAP for reporting genetic variants in cancer. [1-2]

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Microsatellite Instability (MSI)

Microsatellite instability (MSI) is a condition of genetic hypermutability resulting from impaired DNA mismatch repair (MMR) mechanisms. It is characterized by the accumulation of mutations in short repetitive DNA sequences known as microsatellites. MSI-H is found across multiple cancer types, most notably in colorectal, endometrial, gastric, and ovarian cancer. It can arise sporadically or as part of Lynch syndrome, a hereditary condition caused by germline mutations in MMR genes (MLH1, MSH2, MSH6, PMS2).

The results of MSI are divided into three types: MSI-H, which means microsatellites are highly unstable; MSS, which means microsatellites are stable; MSI-U, which means that the sample does not meet the MSI evaluation conditions (tissues or pleural fluid samples may not have passed the MSI indicator calculation quality control due to the low DNA and/or content of tumor cells).

Pembrolizumab has received full FDA approval for adult and pediatric patients with unresectable or metastatic MSI-H or dMMR solid tumors (PMID: 35680043, 33264544) — representing the first full approval for an immunotherapy based on a predictive biomarker, regardless of solid tumor type. This approval applies to patients who have progressed after prior treatment and have no satisfactory alternative options. Beyond the tumor-agnostic indication, specific approvals have been granted for tumor types such as endometrial and colorectal cancer.

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9. Biomarker findings across all Levels of evidence (A-D)

Biomarker	Result	Therapies with strong clinical significance		Therapies with potential significance		Therapies with potential resistance
		Approved/ Standard of care therapies (Level A)	Therapies in well powered studies (Level B)	Off label/ Investigational therapies (Level C)	Therapies in pre-clinical trials (Level D)	
IDH1	Exon 4 c.394C>T (p.R132C) VAF: 8.4%	Ivosidenib	-	Vorasidenib	-	-

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