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ΠΛΗΡΟΦΟΡΙΕΣ

Εξεταζόμενος	-	**Ημ. Συλλογής Δείγμ.**	-
ΑΜΚΑ	-	**Παραπέμπων**	-
Ημερ. Γέννησης	-	**Barcode**	26xxxxxxGR
Τύπος Δείγμ. #1	ΠΛΑΣΜΑ	**Ημερ. Παρ. Δειγμ.**	-
Τύπος Δείγμ. #2	-	**Ημερ. Αποτελ.**	-
Κωδικός Δείγμ. #1	-	**Τύπος όγκου**	Μελάνωμα

Com.Pl.i.t. Dx Liquid Biopsy (21 genes, 9 fusions) | Comprehensive Panel for Individualized Treatment

Αποτελέσματα και ερμηνεία*

Βιοδείκτης	Αποτέλεσμα	Θεραπείες με ισχυρή κλινική σημασία		Θεραπείες με πιθανή κλινική σημασία	Θεραπείες με πιθανή αντίσταση
		Εγκεκριμένες/ βάσει οδηγιών θεραπείες για την ένδειξη (Level A)	Θεραπείες σε ισχυρές μελέτες (Level B)	Εκτός ένδειξης θεραπείες (Level C)	
BRAF	Exon 15 p.V600R VAF: 2.4%	Vemurafenib+Atezolizumab +Cobimetinib Dabrafenib+Trametinib Encorafenib+Binimetinib Vemurafenib+Cobimetinib	-	Tovorafenib	-
Μικροδορυφορική Αστάθεια (MSI)	χωρίς μικροδορυφορική αστάθεια (MSS)	-	-	-	-

*Σημείωση: Το επίπεδο σημαντικότητας των παραλλαγών (Level of Evidence, LoE) (π.χ. A, B, C κλπ) βασίζονται στις οδηγίες για την αναφορά γενετικών παραλλαγών στον καρκίνο που δόθηκαν με κοινή συναίνεση των AMP, ACMG, ASCO και CAP. Για λεπτομερή περιγραφή των οδηγιών αυτών, ανατρέξτε στην Εικόνα 1.

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Γιώργος Νασιούλας, PhD Μοριακός Βιολόγος, Επιστημονικός Διευθυντής, ΑΜΚΑ:26025301255

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SAMPLE INFORMATION			
Name	-	**Date Sp. Extracted**	-
ID/Medical ID	-	**Req. Physician**	-
Date of Birth	-	**Report No**	26xxxxxGR
Material #1	PLASMA	**Date Received**	-
Material #2	-	**Date of Report**	-
Sample #1 ID	-	**Tumor type**	Melanoma

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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)	
BRAF	Exon 15 p.V600R VAF: 2.4%	Vemurafenib+Atezolizumab+Cobimetinib Dabrafenib+Trametinib Encorafenib+Binimetinib Vemurafenib+Cobimetinib	-	Tovorafenib	-
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

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2. Methodology

NGS analysis

ctDNA analysis was performed using plasma-extracted cfDNA. The MagMAX Cell-Free DNA Isolation Kit (Thermofischer Scientific) was used for cfDNA. 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 21 genes includes the entire exon regions and CNVs of 5 genes, partial exon regions of 16 genes and introns/promoters/fusion breakpoint regions of 9 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

21 Gene Alterations									
ARID2	BAP1	BRAF	CDKN2A*	CTNNB1	EIF1AX	ERBB2*	GNA11	GANQ	IDH1
IDH2	KIT	KRAS*	MAP2K1	MAP2K2	NF1	NRAS	PTEN*	SF3B1	TERT
TP53*									
9 Fusion Genes									
ALK	FGFR1	FGFR2	FGFR3	NTRK1	NTRK2	NTRK3	RET	ROS1	
MSI									

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

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%.

Limit of Detection (LoD): The limit of detection (LoD) of this assay is listed in the table below. The LoD is based on as low as 30ng cfDNA input for library preparation.

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

Variant Type	Limit of Detection
Single nucleotide variations (SNV)	VAF ≥0.2%
Insertions/deletions (Indel)	VAF ≥0.2%
Fusion (or rearrangement)	VAF ≥0.5%
Copy number (CNV)	≥2.4 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 cell free tumor DNA (ctDNA) amount could result in false negative results.
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. 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.
6. Improper sample collection, transportation and storage may lead to compromised or invalid results.

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3. Quality Control Results

Quality Control Index		Result	Criterion
Sequencing Quality Assessment	Average effective sequencing depth[1]	**4230**	≥1000
	Fraction of target covered with ≥500x[2]	100%	≥90%
	Fraction of base quality ≥Q30[3]	99%	≥80%
Overall Assessment[4]		PASS	

Note:

1. Average effective sequencing depth: Average sequencing depth on target without duplicated reads.
2. Fraction of target covered with ≥ 500x: The proportion of bases that sequencing depth reach or above 500x on target, this index reflecting the coverage uniformity of sequencing.
3. Fraction of base quality ≥ Q30: The proportion of base quality in sequencing data that reach or above Q30, that is the probability of base recognition accuracy rate exceeds 99.9%.
4. 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	*MSI* status	**MSS**
ERBB2 amplification	Not Detected	*RET* rearrangement	Not Detected
NTRK1/2/3 rearrangement	Not Detected		

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:	*BRAF*: c.1798_1799delGTinsAG (p.V600R)	VAF*: 2.4%	OncoKB

Treatment Information

The RAF-targeted inhibitors encorafenib, dabrafenib and vemurafenib, alone or in combination with the MEK1/2-targeted inhibitors binimetinib, trametinib and cobimetinib respectively, are FDA-approved for the treatment of patients with BRAF V600E/K mutant melanoma and NCCN-compendium listed for the treatment of patients with BRAF V600-mutant melanoma. Additionally, the anti-PD-L1 antibody atezolizumab in combination with cobimetinib + vemurafenib is FDA-approved for the treatment of patients with unresectable or advanced BRAF V600-mutant melanoma.

Gene information

BRAF is a serine/threonine kinase that plays a key role in the regulation of the mitogen-activated protein kinase (MAPK) cascade (PMID: 15520807), which under physiologic conditions regulates the expression of genes involved in cellular functions, including proliferation (PMID: 24202393). Genetic alterations in BRAF are found in a large percentage of melanomas, thyroid cancers and histiocytic neoplasms as well as a small fraction of lung and colorectal cancers. The most common BRAF point mutation is V600E, which deregulates the protein's kinase activity leading to constitutive BRAF activation, as BRAF V600E can signal as a monomer independently of RAS or upstream activation (PMID: 20179705). Other BRAF mutations have been found that affect the protein's propensity to dimerize (PMID: 16858395, 26343582, 12068308). The product of these alterations is a BRAF kinase that can activate MAPK signaling in an unregulated manner and, in some instances, is directly responsible for cancer growth (PMID: 15520807). Inhibitors of mutant BRAF, including vemurafenib and dabrafenib, are FDA-approved for the treatment of late-stage or unresectable melanoma.

Variant Information

The class I activating exon 15 BRAF V600R missense mutation is located in the kinase domain of the BRAF protein (PMID: 28783719). BRAF V600R is the third most common mutation occurring in 5-7% of patients with BRAF-mutant melanoma (PMID: 22536370, 23237741, 22614978). Comprehensive biological characterization of the BRAF V600R mutation has demonstrated that this mutation activates the downstream MAPK pathway independently of RAS and renders BRAF constitutively activated in monomeric form (PMID: 15035987, 28783719, 26343582, 20179705). Preclinical studies with melanoma cells expressing BRAF V600R demonstrate sensitivity to treatment with AZ628, TAK-632, PLX7904, vemurafenib and dabrafenib as measured by inhibition of cellular proliferation (PMID: 27523909). In a case report, a patient with melanoma harboring BRAF V600R was treated with a combination therapy of dabrafenib plus trametinib and demonstrated partial response with a progression-free survival of 52.2 months (PMID: 36039514). In another case report, a patient with melanoma harboring BRAF V600R was treated with dabrafenib and demonstrated partial response with lesion reduction and progression-free survival of seven months (PMID: 27255157). The BRAF V600R mutation is known to be pathogenic.

Vemurafenib+Atezolizumab+Cobimetinib DrugBank

The combination of vemurafenib, an inhibitor of V600-mutant BRAF, and cobimetinib, an inhibitor of MEK1/2, with atezolizumab, an immunotherapeutic PD-L1 antibody, is FDA-approved for patients with BRAF V600 mutation-positive unresectable or

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metastatic melanoma. FDA approval was based on the results of the Phase III double-blind, randomized, placebo-controlled IMspire150 trial of Atezolizumab + Cobimetinib + Vemurafenib versus Placebo + Cobimetinib + Vemurafenib in 514 patients with BRAF V600-mutant melanoma in which the median progression-free survival was 15.1 mos (95% CI=11.4,18.4) in the triplet arm versus 10.6 mos (95% CI=9.3,12.7) in the doublet + placebo arm (HR=0.78; 95% CI= 0.63, 0.97; p=0.0249) (PMID: 32534646).

Dabrafenib+Trametinib

DrugBank

Dabrafenib, an orally bioavailable RAF inhibitor, and trametinib, an orally bioavailable MEK1/2 inhibitor, are FDA-approved alone or in combination for the treatment of patients with metastatic melanoma harboring a V600E or V600K BRAF mutation. FDA approval of dabrafenib in combination with trametinib was based on results from an open-label Phase III study of combination therapy versus dabrafenib monotherapy in 247 patients with metastatic melanoma who were naive to treatment with BRAF inhibitors. Combined dabrafenib and trametinib, administered in full monotherapy doses, improved the response rate in patients with BRAF V600-mutant metastatic melanoma versus dabrafenib monotherapy (67% vs.51%; p<0.002). However, median progression-free survival improved by only two weeks (9.3 months vs 8.8 months; HR = 0.75) compared with dabrafenib monotherapy (PMID: 23020132). Combination therapy is associated with less cutaneous toxicity than monotherapy, but systemic toxicity may be increased (PMID: 25287827, 25399551). Follow-up trials have demonstrated that all clinical measures inclusive of overall and median progression-free survival as well as objective response rates, median duration of response and number of patients with complete response favored patients treated with combination dabrafenib and trametinib, administered in full monotherapy doses, compared to either dabrafenib or vemurafenib monotherapy, including patients who previously progressed on BRAF inhibitor monotherapy (PMID: 25287827, 25399551, 25265492). Additionally, patients with melanoma treated with dabrafenib and trametinib in both the neoadjuvant and adjuvant settings have improved survival over patients given standard of care (PMID: 29361468, 28991513, 28891408). Promising clinical data has also suggested that addition of an immunotherapy agent to combination RAF and MEK inhibitor treatment may improve rate of overall response and duration of response in melanoma patients (PMID: 31171876, 31171879, 31171878).

Encorafenib+Binimetinib

DrugBank

The combination of encorafenib, an inhibitor of V600E- or V600K-mutant BRAF, and binimetinib, an inhibitor of MEK1/2, is FDA-approved in combination for patients with unresectable or metastatic melanoma with a BRAF V600E or V600K mutation. FDA approval was based on the results of the Phase III COLUMBUS trial of combined encorafenib plus binimetinib versus single agent vemurafenib in 577 patients with BRAF V600E- or V600K-mutant metastatic melanoma in which the median progression-free survival was 14.9 months (95% CI: 11.0-18.5) in the encorafenib plus binimetinib group versus 7.3 months (95% CI: 5.6-8.2) in the single agent vemurafenib group (HR= 0.54, 95% CI: 0.41-0.71; p<0·0001) (PMID: 29573941).

Vemurafenib+Cobimetinib

DrugBank

The combination of vemurafenib, an orally available kinse inhibitor of V600-mutant BRAF, and cobimetinib, an orally available kinase inhibitor of MEK1/2, is FDA-approved for the treatment of patients with BRAF V600-mutant metastatic or unresectable locally advanced melanoma. FDA approval was based on results from the randomized Phase III coBRIM trial of combined vemurafenib and cobimetinib versus vemurafenib and placebo in 495 patients with metastatic BRAF V600-mutant melanoma that demonstrated superior

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clinical benefit measures in the combination versus the control arm. Specifically, median progression-free survival was 9.9 months in the combination arm versus 6.2 months in the control arm (HR = 0.51), with a complete response rate of 10% versus 4%, respectively, and interim nine-month overall survival of 81% versus 73%, respectively (PMID: 25265494). Follow-up analysis of the coBRIM trial showed two-year overall survival was 48.3% in the combination group versus 38.0% in the monotherapy group (PMID: 27480103).

Tovorafenib DrugBank

Tovorafenib is an orally available, pan-RAF small molecule inhibitor that is FDA-approved for the treatment of pediatric patients six months of age and older with relapsed or refractory pediatric low-grade glioma (LGG) harboring a BRAF fusion or rearrangement, or BRAF V600 mutation. FDA approval was based on the results of the Phase II FIREFLY-1 (NCT04775485) trial of tovorafenib in 76 patients (median age=8 years old [range=2-21]) with relapsed or refractory pediatric LGG harboring an activating BRAF alteration based on local laboratory testing. In the Phase II FIREFLY-1 (NCT04775485) trial, the overall RAPNO-LGG cohort demonstrated an objective response rate (ORR) of 51% (95% CI=40-63), with a 37% (n=28) partial response rate and 14% (n=11) minor response rate, a median duration of response (DOR) of 13.8 months (95% CI=11.3-NE) in 39 patients and a median progression-free survival of 13.8 months (95% CI=8.3-16.9) (PMID: 37978284). The BRAF V600E mutation subcohort (n=12) demonstrated an ORR of 50% (95% CI=21-79) and a median DOR that was not evaluable (95% CI=8.4-NE) (PMID: 37978284).

*VAF: Variant Allele Frequency

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7. Clinical Trials to consider

BRAF associated clinical trials

NCT03340506	PHASE4
Title	Dabrafenib and/or Trametinib Rollover Study
Treatment	dabrafenib | trametinib
Location	Argentina, Austria, China, Denmark, France, Germany, Hungary, Japan, Netherlands, Spain, Thailand, United States

NCT05727904	PHASE3
Title	Study to Investigate Lifileucel Regimen Plus Pembrolizumab Compared With Pembrolizumab Alone in Participants With Untreated Advanced Melanoma.
Treatment	Lifileucel plus Pembrolizumab | Pembrolizumab with Optional Crossover Period
Location	Australia, Belgium, Canada, France, Germany, Israel, Italy, Netherlands, South Korea, Spain, Sweden, Switzerland, United Kingdom, United States

NCT03563729	PHASE2
Title	Melanoma Metastasized to the Brain and Steroids
Treatment	Pembrolizumab Injection [Keytruda] | Ipilimumab Injection [Yervoy] | Nivolumab Injection [Opdivo] | Encorafenib | Binimetinib | Dabrafenib | Trametinib
Location	Denmark

NCT04116541	PHASE2
Title	A Study Evaluating the Activity of Anti-cancer Treatments Targeting Tumor Molecular Alterations/Characteristics in Advanced / Metastatic Tumors.
Treatment	HDM201 | Ribociclib | Cabozantinib | Alectinib | Regorafenib | Trametinib | Dabrafenib | Avapritinib
Location	France

NCT05503797	PHASE2

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Name	-	Report No	26xxxxxxGR

Title	A Study to Assess the Efficacy and Safety of FORE8394 in Participants With Cancer Harboring BRAF Alterations
Treatment	Plixorafenib
Location	Australia, Canada, France, Germany, Italy, Norway, South Korea, Spain, Sweden, United Kingdom, United States

NCT06703398	**PHASE2**
Title	A Study of GC101 TIL in Advanced Melanoma
Treatment	GC101 TIL | dacarbazine, temozolomide, paclitaxel ,platinum or cisplatin
Location	China

NCT03645928	**PHASE2**
Title	Study of Autologous Tumor Infiltrating Lymphocytes in Patients With Solid Tumors
Treatment	Lifileucel | LN-145 | Pembrolizumab | LN-145-S1 | Ipilimumab | Nivolumab | Nivolumab-relatlimab | Cisplatin | Carboplatin | Paclitaxel | Nab-Paclitaxel | Pemetrexed
Location	Canada, France, Germany, Greece, Spain, Switzerland, United Kingdom, United States

NCT06906822	**PHASE2**
Title	PLUG-IN: Pembrolizumab Combined With Enfortumab Vedotin for Advanced Melanoma Patients
Treatment	enfortumab vedotin | pembrolizumab
Location	Spain

NCT05355701	**PHASE1**
Title	A Study to Learn About the Study Medicine Called PF-07799933 in People With Advanced Solid Tumors With BRAF Alterations.
Treatment	PF-07799933 | binimetinib | cetuximab | midazolam | fluorouracil | leucovorin | oxaliplatin
Location	Canada, Israel, United States

NCT05538130	**PHASE1**

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Name	-	Report No	26xxxxxxGR

Title	A Study to Learn About the Study Medicine Called PF-07799544 as Monotherapy or in Combination in People With Advanced Solid Tumors
Treatment	PF-07799544 | PF-07799933
Location	Australia, Brazil, Canada, China, Israel, Japan, United States

NCT03743298	**PHASE1**
Title	Safety of AV-MEL-1 With Anti-PD-1 Therapy in Metastatic Melanoma
Treatment	AV-MEL-1
Location	United States

NCT04741997	**EARLY_PHASE1**
Title	Adjuvant Therapy Based on Pathologic Response After Neoadjuvant Encorafenib Binimetinib in Melanoma
Treatment	Encorafenib Pill | Binimetinib Pill | Nivolumab
Location	United States

NCT04903119	**PHASE1**
Title	Nilotinib Plus Dabrafenib/Trametinib or Encorafenib/Binimetinib in Metastatic Melanoma
Treatment	Nilotinib 100mg | Nilotinib 200mg | Nilotinib 300mg | Nilotinib 400mg | Dabrafenib | Trametinib | Encorafenib | Binimetinib
Location	United States

Press here for a live search of clinical trials for BRAF

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8. Appendix

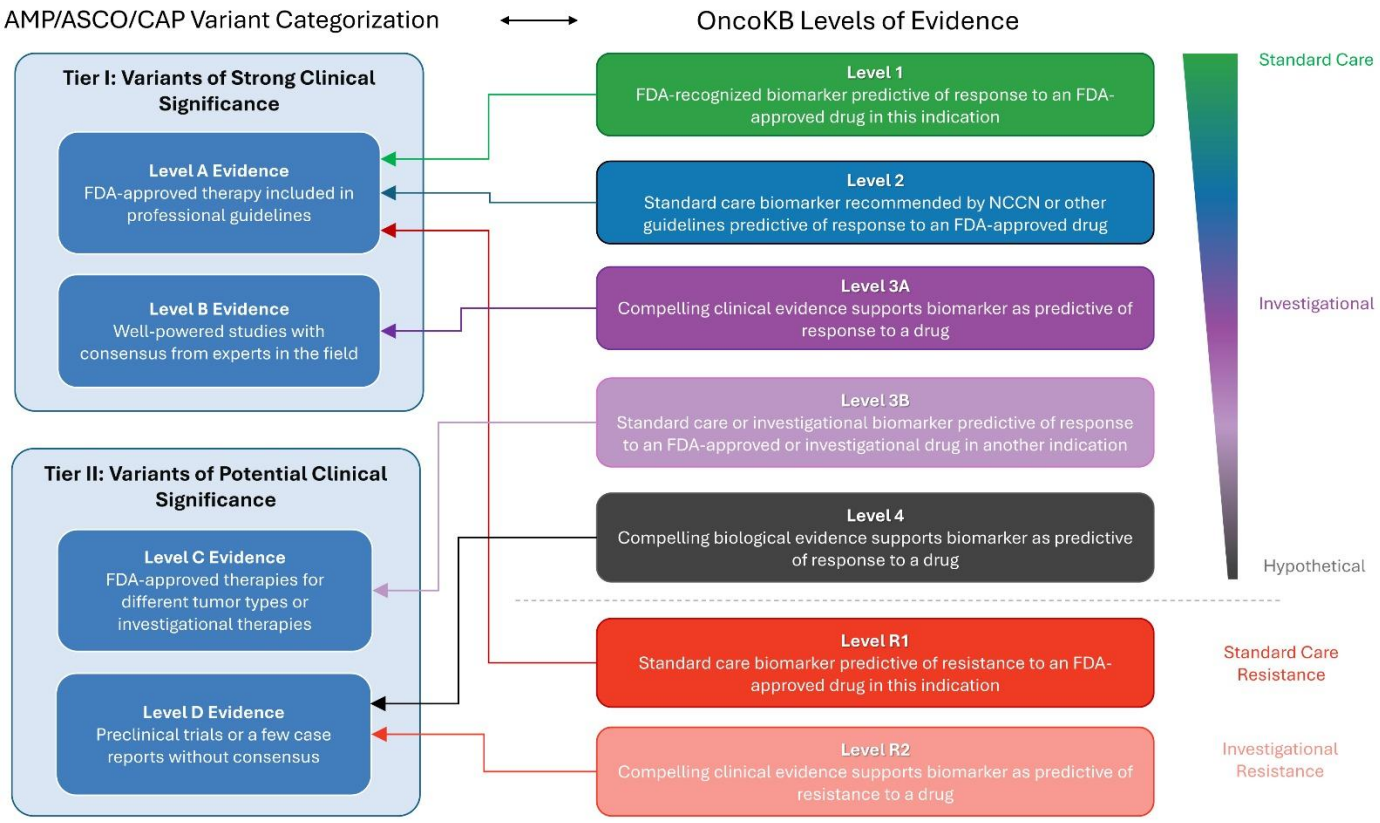

Figure 1. Joint consensus recommendation of AMP, ACMG, ASCO and CAP for reporting genetic variants in cancer. [1-2]

1. Li MM, Datto M, Duncavage EJ, et al. J Mol Diagn. 2017 Jan;19(1):4-23.
2. Leichsenring J, Horak P, Kreutzfeldt S, et al. Int J Cancer. 2019 Dec 1;145(11):2996-3010.

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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)	
BRAF	Exon 15 c.1798_1799del GTinsAG (p.V600R) VAF: 2.4%	Vemurafenib+Atezolizumab +Cobimetinib Dabrafenib+Trametinib Encorafenib+Binimetinib Vemurafenib+Cobimetinib	-	Tovorafenib	-	-

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10. References

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