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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**	GIST

Com.Pl.i.t. Dx Liquid Biopsy (17 genes, 9 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)	
PDGFRA	Exon 18 p.D842V VAF: 0.3%	Avapritinib Dasatinib Regorafenib Ripretinib Sunitinib	-	-	Imatinib
KIT	Exon 11 p.V559_E561del VAF: 0.4%	Imatinib Sunitinib Regorafenib Ripretinib	-	-	-
RB1	Exon 8 p.R255* VAF: 2.1%	-	-	-	-
RB1	Exon 23 p.P781Qfs*29 VAF: 0.5%	-	-	-	-
TP53	Exon 7 p.S261Vfs*84 VAF: 0.6%	-	-	-	-
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.

52, Spaton Ave., 15344, Gerakas, Greece, G.E.MI. nr: 0007856001000 | info@genekor.com, www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
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George Nasioulas, PhD Molecular Biologist, Scientific Director, Medical ID:26025301255

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2. Methodology

DNA was extracted from the sample under investigation using the MagMax Total Nucleic Acid Isolation Kit (ThermoFisher). A capture based targeted next generation sequencing (NGS) analysis was performed, using the Oncology Multi-Gene Variant Assay (GenePlus) which is a qualitative in vitro diagnostic test. The analysis includes the entire exon regions and CNVs of 17 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

17 Gene Alterations									
ARID1A*	ATRX*	BRAF*	ERBB2*	KIT*	KRAS*	NF1*	PDGFRA*	PIK3CA*	PTEN*
RB1*	SDHA*	SDHAF2*	SDHB*	SDHC*	SDHD*	TP53*			
9 Fusion Genes									
ALK	FGFR1	FGFR2	FGFR3	NTRK1	NTRK2	NTRK3	RET	ROS1	
MSI									

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

Biomarkers within the scope of accreditation (ISO15189:2022)

- *BRAF, KRAS, PIK3CA, PTEN* (SNV/indel detection by 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 100%.
Specificity: Negative reference standards are tested with the assay, and the negative percent agreement (NPA) of SNVs, Indels, fusions and CNVs was 100%.
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 50 ng of gDNA input for library preparation. The assay can also be used to test microsatellite instability (MSI) with a tumor cell content as low as 10%.

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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 ≥0.3%
Insertions/deletions (Indel)	VAF ≥0.3%
Fusion (or rearrangement)	VAF ≥0.5%

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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Quality Control Index		Result	Criterion
Sequencing Quality Assessment	Average effective sequencing depth[1]	**3031**	≥10000
	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 with duplicated reads.
2. Fraction of target covered with ≥ 500x: The proportion of bases that sequencing depth reaches 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	*PDGFRA* mutation	**p.D842V (0.3%)**
KIT mutation	**p.V559_E561del (0.4%)**	*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	**Detected**	**May increase the benefit rate of PD-1/PD-L1 inhibitors**
Treatment effect - negative correlation		
ALK rearrangement	Not Detected	-
PTEN inactivating mutation	Not Detected	-

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6. Interpretations for targeted therapies

Genetic Variation:	*PDGFRA*: c.2525A>T (p.D842V)	VAF*: 0.3%	OncoKB

Treatment Information

While patients with PDGFRA D842V mutant gastrointestinal stromal tumors (GIST) do not respond to imatinib, the kinase inhibitor avapritinib is FDA-approved for the treatment of patients with PDGFRA D842V mutant GIST. The multikinase inhibitors sunitinib, regorafenib and ripretinib are FDA-approved as second-, third- and fourth-line therapies respectively for progressive disease after imatinib.

Gene information

The PDGFRA gene encodes for the protein Platelet-derived growth factor alpha (PDGFRA). Binding of ligand to the extracellular domain of PDGFRA, which contains immunoglobulin (Ig)-like domains, causes dimerization followed by autophosphorylation of the receptor and activation of downstream pathways such as RAS-MAPK, PI3K and PLC-γ that are involved in developmental and cellular responses. The catalytic activity of PDGFRA is mediated through the split intracellular tyrosine kinase domain; PDGFRA binds to all PDGF ligand isoforms except PDGF-DD (PMID: 18483217, 24703957). Mutations, insertions, deletions, fusions and genomic amplification of PDGFRA lead to its activation in several tumor types: ~7% of gastrointestinal stromal tumors (GISTs) have PDGFRA activating mutations and these mutations are mutually exclusive from KIT mutations (PMID: 20023271); activating mutations in PDGFRA have been been reported in ~5% of Chinese melanoma patients (PMID: 24132921); amplification of PDGFRA is the second most frequent receptor tyrosine kinase amplification in glioblastoma (GBM), is found in ~7-15% of GBM tumors and is often associated with in-frame deletions (PMID: 18772890, 19915670, 22323597, 20129251, 20889717); amplification of the PDGFRA locus has been reported in more than 80% of intimal sarcomas (PMID: 20685895), ~19% of malignant peripheral nerve sheath tumors (PMID: 16357008), 3-7% of non-small cell lung adenocarcinomas and 8-10% non-small cell lung squamous cell carcinomas (PMID: 19755855); activating mutations have been found in ~5% of diffuse intrinsic pontine gliomas and in ~14% of non-brain stem pediatric high-grade gliomas, around 40% of these occurring in the context of PDGFRA amplification (PMID: 23970477); chimeric fusion transcripts to the catalytic domain of PDGFRA have been reported in select cases of GBM (PMID: 20889717), chronic myeloid leukemia (PMID: 12023981, 12944919, 15034867) and hypereosinophilic syndrome (HES)/chronic eosinophilic leukemia (CEL) (PMID: 12660384, 19187542, 16498388, 16845659, 17555450). PDGFRA mutations have also been reported in inflammatory fibroid polyps (PMID: 22394371), and these mutations have been characterized as activating in other disease types.

Variant Information

The PDGFRA D842V mutation occurs in the PDGFRA tyrosine kinase domain. This mutation has been found in patients with gastrointestinal stromal tumors. Expression of the PDGFRA D842V mutation in hematopoietic and epithelial cells as well as in mouse models demonstrated that it is activating and oncogenic as shown by increased pathway activation, proliferation, and the development of gastrointestinal hyperplasia and gliomas. In vitro experiments involving cell lines and mouse models expressing this mutation show that it is not sensitive to the kinase inhibitor, imatinib, as evidenced by sustained signaling and proliferation in the presence of the drug (PMID: 24132921, 16030188, 22665524, 15928335, 23970477, 18794084, 22745105, 15685537, 18955458, 23752188, 12522257, 17087936, 19217431, 12949711). The PDGFRA D842V mutation is known to be pathogenic.

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Imatinib

DrugBank

Imatinib, a small molecule inhibitor of KIT, PDGFRA and other kinases, is FDA-approved for adult patients with gastrointestinal stromal tumors (GISTs). The NCCN lists PDGFRA D842V as a mutation that has known insensitivity to imatinib. In vitro studies in hematopoietic and epithelial cells engineered to express the PDGFRA D842V alteration were resistant to imatinib treatment, as evidenced by signaling, proliferation, and apoptosis assays (PMID: 15928335, 12949711, 22718859, 18794084, 22745105, 15685537, 18955458, 25905001, 23752188). Mouse models and gastrointestinal stromal tumor (GIST) patient samples with the PDGFRA D842V mutation demonstrated resistance to imatinib therapy (PMID: 17087936, 18794084). In an international survey among GIST referral centers, 31 of 58 patients with PDGFRA alterations had the PDGFRA D842V mutation and none of the 31 patients achieved a clinical response to imatinib (PMID: 22718859). Additional studies have demonstrated that GIST patients with the PDGFRA D842V mutation are resistant to imatinib therapy (PMID: 18955458, 25905001). In a study of 26 GIST patients, one individual treated with imatinib developed secondary resistance and no longer responded to imatinib therapy (PMID: 15685537). In a randomized phase II clinical study of imatinib therapy in advanced GIST, two of 48 patients with imatinib-resistant primary disease had a PDGFRA D842V alteration. Patients with the PDGFRA D842V alteration should consider alternative tyrosine kinase inhibitor therapies.

Avapritinib

DrugBank

Avapritinib is an orally available, small molecule inhibitor of PDGFRA D842V and KIT D816 mutant kinases that is FDA-approved for the treatment of adults with unresectable or metastatic gastrointestinal stromal tumor (GIST) harboring a PDGFRA exon 18 mutation, including PDGFRA D842V mutations. FDA approval was based on the Phase I NAVIGATOR (NCT02508532) trial of avapritinib in 43 patients with PDGFRA exon 18-mutant (PDGFRA D842V/Y, D842_H845del, D842_H845insV) GIST.

In the Phase I NAVIGATOR (NCT02508532) trial, the PDGFRA exon 18-mutant cohort demonstrated an overall response rate (ORR) of 84% (95% CI=69-93), including three complete responses (CR) and 33 partial responses (PR), and a duration of response (DOR) that was greater than six months in 22 patients (61%) (PMID: 32615108). In the subpopulation with PDGFRA D842V mutations (n=38/43 patients), the ORR was 89% (95% CI=75-97), including three CRs and 31 PRs, and the DOR was greater than six months in 20 patients (59%) (PMID: 32615108).

Dasatinib

DrugBank

Dasatinib, a small molecule inhibitor of KIT, PDGFRA and other kinases, is FDA-approved for BCR-ABL-positive chronic myeloid leukemia and acute lymphoblastic leukemia and is an NCCN category 2A recommendation for patients with PDGFRA D842V-mutant gastrointestinal stromal tumor (GIST). NCCN recommendation is based on the results of a Phase II trial of dasatinib in 50 patients with GIST who progressed on imatinib in which median progression-free survival and median overall survival were two and nineteen months, respectively (Abstract: Trent et al. Abstract# 10006, ASCO 2011. http://meetinglibrary.asco.org/content/79120-102). Of the fifteen patients whose genotypes were available for analysis, six had KIT exon 11 mutations, two had KIT exon 9 mutations, one had PDGFR D842V mutation, and six were wildtype for KIT and PDGFR (Abstract: Trent et al. Abstract# 10006, ASCO 2011. http://meetinglibrary.asco.org/content/79120-102).

Regorafenib

DrugBank

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Regorafenib, a small molecule inhibitor of KIT and other kinases (e.g. VEGFR2 and -3, PDGFR), is FDA-approved for patients with locally advanced, unresectable or metastatic gastrointestinal stromal tumor (GIST) who have been previously treated with imatinib mesylate and sunitinib malate. In a randomized, double-blind, placebo-controlled, multicenter, phase III trial (NCT01271712) of 199 patients with imatinib and sunitinib-resistant, metastatic GIST that received regorafenib (n=133 patients) or placebo (n=66 patients), median progression-free survival (PFS) per independent blinded central review was 4.8 months (IQR 1.4–9.2) for regorafenib and 0.9 months (0.9–1.8) for placebo (HR= 0.27; 95% CI= 0.19–0.39; p<0·0001) and following progression, 56/66 patients (84.8%) on placebo crossed over to regorafenib, resulting in no significant difference in overall survival (OS) between study arms (HR= 0.77; 95% CI= 0.42–1.41; p=0.199) (PMID: 23177515). In a multicenter, open-label, phase II trial (NCT01068769) of regorafenib in 33 adult patients with imatinib- and sunitinib-resistant metastatic GIST, the clinical benefit rate was documented in 25/33 patients (76%; 95% CI= 58-89), including six partial responses; the median PFS was 13.2 months (95% CI= 9.2–18.3 months), including four patients who remained progression-free at study closure, each achieving clinical benefit for more than three years (range 36.8–43.5 months); and the median OS was 25 months (95% CI= 13.2–39.1 months) (PMID: 27371698).

Ripretinib

DrugBank

Ripretinib is a small molecule inhibitor of KIT and PDGFRA kinases that is FDA-approved for adult patients with advanced gastrointestinal stromal tumors (GIST) who have received prior treatment with three or more kinase inhibitors. FDA approval was based on the results of the international, multi-center, randomized (2:1), double-blind, placebo-controlled trial (NCT03353753) of ripretinib in 129 patients with advanced, pretreated GIST in which the overall response rate was 9% (95% CI= 4.2, 18), the median progression-free survival was 6.3 months (95% CI= 4.6, 6.9), and the median overall survival was 15.1 months (95% CI= 12.3, 15.1) (PMID: 32511981).

Sunitinib

DrugBank

Sunitinib is a small molecule inhibitor of multiple receptor tyrosine kinases that is NCCN-listed and FDA-approved for patients with gastrointestinal stromal tumor (GIST) after disease progression on or intolerance to imatinib mesylate. In a randomized, double-blind, placebo-controlled, multicenter, international, phase III trial (NCT00075218) of 312 patients with advanced imatinib-resistant/intolerant GIST who received either sunitinib (n=207 patients) or placebo (n=105 patients), the median time to tumor progression was 27.3 weeks (95% CI= 16.0–32.1) for patients receiving sunitinib and 6.4 weeks (95% CI= 4.4–10.0) for those on placebo (HR 0.33; p<0·0001) (PMID: 17046465). In a randomized, phase II study (NCT00137449) of sunitinib in 60 patients with imatinib-resistant/intolerant GIST, the clinical benefit rate was 53% (95% CI= 40–66), eight patients (13%) achieved objective partial responses, and 24 (40%) achieved stable disease ⩾24 weeks (PMID: 19282169). In a worldwide treatment-use trial of sunitinib in 1,124 patients with imatinib-resistant/intolerant GIST, the median treatment duration was 7.0 months, the median time to tumor progression was 8.3 months (95% CI= 8.0–9.4), and median overall survival was 16.6 months (95% CI= 14.9–18.0), with 36% of patients alive at the time of analysis (PMID: 25641662).

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Genetic Variation:	*KIT*: c.1676_1684del (p.V559_E561del)	VAF*: 0.4%	OncoKB

Treatment Information

The multikinase KIT and PDGFRA inhibitors imatinib, sunitinib, regorafenib and ripretinib are FDA-approved for the treatment of patients with gastrointestinal stromal tumors (GISTs) harboring KIT exon 11 alterations, such as KIT V559_E561del.

Gene information

The proto-oncogene KIT encodes a type 3 transmembrane receptor tyrosine kinase. The receptor is activated through dimerization and autophosphorylation upon binding by its ligand, stem cell factor (SCF) also known as mast cell growth factor (MGF) (PMID: 9438854). KIT activation results in increased intracellular signaling through several pathways including PI3K, MAPK and STAT, ultimately leading to cell proliferation and survival (PMID: 17546049, 11896121, 22089421). For patients with wildtype gastrointestinal stromal tumors (GIST; no KIT or PDGFRA mutations), NCCN recommends testing for germline succinate dehydrogenase (SDH) mutations. About 10-15% of GISTs are wildtype; thus, the absence of a mutation does not exclude the diagnosis of GIST. In patients without KIT mutations, a subset of those with advanced GISTs benefit from imatinib (0-45% of patients) (NCCN Soft Tissue Sarcoma v.2.2017). Activating KIT mutations occur in 80 - 90% of GISTs and are distributed over multiple exons with different frequencies (exons 11 (66.1%), exon 9 (13%), exon 13 (1.2%), and exon 17 (0.6%)) (PMID: 15365079, 17268243, 11719439). There are at least eight small molecule tyrosine kinase inhibitors (TKIs) targeting KIT that have been approved by the US Food and Drug Administration with the efficacy of each TKI strongly depending on the location of the activating KIT mutation (PMID: 2427414, 18955458, 19164557).

Variant Information

A similar mutation, KIT V559_V560del, is located in the juxtamembrane domain in exon 11 of the protein. This mutation has been found in gastrointestinal stromal tumors (GIST) (PMID: 16551858) and hematopoietic malignancies (PMID: 15790786). Biological characterization of Ba/F3 hematopoietic cells expressing this variant demonstrated that this mutation is activating and promotes growth, as determined by pathway activation and factor-independent growth (PMID: 15790786). In vitro studies have suggested that this mutation is sensitive to imatinib, as evidenced by pathway activation and cell viability (PMID: 15790786). In a phase II study of 147 patients with advanced, unresectable GIST treated with imatinib, one individual with KIT V559_V560del responded to imatinib before acquiring secondary resistance (PMID: 16954519). The KIT V559_E561del alteration is known to be Pathogenic.

Imatinib DrugBank

Imatinib is a small molecule inhibitor of KIT and other kinases that is FDA-approved for adult patients with gastrointestinal stromal tumors (GISTs). FDA approval was based on the Phase II trial of imatinib in 147 patients with malignant or unresectable GIST in which 56 patients across two dose levels had a partial response, giving an overall response rate of 38% (95% CI= 30-46) (PMID: 12181401). Two further Phase III studies of imatinib in 946 or 694 patients, respectively, with KIT-expressing malignant or unresectable GIST demonstrated a greater response rate in patients given 800mg imatinib versus 400mg, with complete response rates ranging from 44.6%-48.1% at the 800 mg dose versus 5.2%-5.3% at the 400mg dose (PMID: 18235122, 18955451). Long-term follow-up of these patients demonstrated a ten-year overall survival across both study arms of 22% (95% CI= 19.0-26.0), with patients harboring KIT exon 9 mutations having significantly shorter overall survival on imatinib than patients with KIT exon 11 mutations (p=0.0013) (PMID: 28196207).

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Sunitinib

DrugBank

Sunitinib is a small molecule inhibitor of KIT and other kinases that is FDA-approved for use in gastrointestinal stromal tumors (GIST) after disease progression on or intolerance to imatinib mesylate. FDA approval was based on the randomized, double-blind Phase III study of sunitinib versus placebo in 312 patients with imatinib-resistant, unresectable GIST in which median time to progression was 27.3 weeks versus 6.4 weeks (HR = 0.33; p<0.0001) in the sunitinib versus placebo groups, respectively (PMID: 17046465). In the sunitinib group, 7% (n=14), 58% (n=120) and 19% (n=39) of patients had a partial response, stable disease or progressive disease, while 0%, 48% (n=50) and 37% (n=39) of patients in the placebo group had a partial response, stable disease or progressive disease, respectively (PMID: 17046465).

Regorafenib

DrugBank

Regorafenib is a small molecule inhibitor of KIT and other kinases (e.g. VEGFR2 and -3, PDGFR) that is FDA-approved for patients with advanced, unresectable gastrointestinal stromal tumors (GIST) that no longer respond to imatinib and sunitinib. FDA approval was based on results from the randomized, double-blind Phase III trial of regorafenib versus placebo in 199 patients with unresectable GIST that progressed on imatinib and sunitinib in which the progression-free survival was 4.8 months on regorafenib versus 0.9 months on placebo (HR=0.77; p < 0.0001) and the disease control rates were 53% and 9%, respectively (PMID: 23177515). For patients with KIT exon 11 (n=51) or exon 9 (n=15) mutations, the hazard ratios were 0.212 (95% CI =0.098-0.458) and 0.239 (95% CI= 0.065-0.876), respectively, in favor of regorafenib (PMID: 23177515).

Ripretinib

DrugBank

Ripretinib is a small molecule inhibitor of KIT and PDGFRA kinases that is FDA-approved for adult patients with advanced gastrointestinal stromal tumors (GIST) who have received prior treatment with three or more kinase inhibitors. FDA approval was based on the results of the international, multi-center, randomized (2:1), double-blind, placebo-controlled trial (NCT03353753) of ripretinib in 129 patients with advanced, pretreated GIST in which the overall response rate was 9% (95% CI= 4.2, 18), the median progression-free survival was 6.3 months (95% CI= 4.6, 6.9), and the median overall survival was 15.1 months (95% CI= 12.3, 15.1) (PMID: 32511981).

Genetic Variation:	*RB1*: c.763C>T (p.R255*)	VAF*: 2.1%	OncoKB

Gene information

RB1, also known as RB, is involved in the cell-cycle checkpoint and in its active form inhibits the transition from G1 to S phase of the cell cycle until the cell is ready to divide. RB is active in its unphosphorylated form where it binds to E2F family of transcription factors, which together with the E2F Dimerization Partner (E2F-DP), inhibits the transcription of S-phase promoting factors by recruiting histone deacetylases (HDACs) and induce heterochromatin formation (PMID: 1655277). At the end of G1, cyclin-dependent kinases (CDKs) phosphorylate RB to pRB which leads to its dissociation from the E2F-DP complex, thereby allowing entry into S-phase. RB remains phosphorylated until the end of mitosis at which point it is dephosphorylated by protein phosphatase 1 (PP1) to activate the G1-S-phase checkpoint (PMID: 20694007). In addition to its role in G1 cell cycle arrest, RB1 has also been shown to play a role in safeguarding genome stability and mediating apoptosis, senescence and differentiation in response to various stimuli (PMID:

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22293180). As a result of its role in these essential cellular functions, loss of function of RB1 not only leads to unregulated cell division and growth but also to the abrogation of multiple mechanisms that safeguard against cellular transformation and tumorigenesis. Loss-of-function and deletions of RB1 have been associated with many human cancers including lung, breast, prostate and bladder cancers, and concomitant loss of RB1 and p53 are thought to constitute a tumor-initiating event (PMID: 12204530). Homozygous loss or inactivation of the RB1 gene is a hallmark of retinoblastoma (PMID: 22293180), and heterozygous germline mutations in RB1 predispose children to retinoblastoma (PMID: 10502774, 24688104, 27068507) and adults to sarcoma and other tumors (PMID: 16269091, 22205104).

Variant Information

RB1 truncating mutations produce several forms of C-terminally truncated RB1 proteins. These mutations have been found as germline mutations in familial retinoblastoma (PMID: 14769601). Loss of RB1 in an osteosarcoma cell line resulted in increased genome instability, DNA damage and tumor growth in a xenograft model compared to wildtype (PMID: 31138663). Loss of RB1 in germinal center B-cells of an in-vivo mouse model induced hyperproliferation of splenic B cells that eventually resulted in increased cell death compared to wildtype (PMID: 26607597). Six patients with ER+HER2- advanced breast cancer harboring RB1 mutation, out of a study of 127 patients, demonstrated resistance to the CDK4/6 inhibitor palbociclib in combination with the ESR1 inhibitor fulvestrant (PMID: 30206110). Truncating mutations of RB1 often predispose patients who have been successfully treated for hereditary retinoblastoma to secondary malignancies (PMID: 22205104). The RB1 R255* is a truncating mutation in a tumor suppressor gene, and therefore is likely pathogenic. There are no FDA-approved or NCCN-compendium listed treatments specifically for patients with RB1 P781Qfs*29 mutant gastrointestinal stromal tumors.

Genetic Variation:	***RB1*: c.2342del (p.P781Qfs*29)**	**VAF*: 0.5%**	**OncoKB**

Variant Information

The RB1 P781Qfs*29 is another truncating mutation, therefore it is considered to be likely pathogenic, as well.

Genetic Variation:	***TP53*: c.780del (p.S261Vfs*84)**	**VAF*: 0.6%**	**OncoKB**

Gene information

TP53 encodes the p53 tumor suppressor protein, a transcription factor that responds to cellular stresses, including DNA damage and oncogenic activation, by inducing downstream anti-tumor responses such as DNA repair and apoptosis (PMID: 11099028). p53 levels are kept low in healthy cells due to negative regulation by MDM2/4, Cop1 and Trim24 and constant degradation by the ubiquitin-proteasome system (PMID: 36859359, 36207426). When DNA is damaged, a network of pathways is activated to detect and repair lesions in a cell- and context-specific manner (PMID: 36207426). p53 is rapidly phosphorylated by upstream regulators such as ATM, ATR, and CHL1/2, which results in the accumulation of stable p53 (PMID: 36207426). p53 then binds to specific DNA sequences to direct the expression of a wide variety of genes, including those involved in apoptosis, cell cycle arrest, DNA repair, senescence, stem cell differentiation, autophagy, cellular metabolism, and others (PMID: 27141080, 36859359, 36207426). Oncogenic mutations of TP53 often result in the dysregulation of p53 function, usually due to structural changes in the DNA binding domain (PMID: 36859359). Loss

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of p53 function can have various outcomes including tumorigenesis, invasion and metastasis, drug resistance, metabolic reprogramming, immune evasion and overall genomic instability (PMID: 36859359). TP53 is the most commonly mutated gene in human cancers, and germline mutations occur in the cancer predisposition syndrome Li-Fraumeni (PMID: 22713868, 21765642). Clinical and preclinical research into drugs that target TP53 is ongoing, notably with MDM2 inhibitors that aim to restore p53 function and are being tested in combination with other cancer therapies (PMID: 36859359, 37818252).

Variant Information

Truncating mutations of TP53 occur throughout the gene and lead to the production of several C-terminally truncated protein forms. These alterations are predicted to be inactivating and are associated with poor prognosis (PMID: 11900253, 11753428, 16007150, 21467160, 19336573). Experimental studies have revealed that truncating mutations promote cancer cell proliferation, survival and metastasis, since ectopic expression of these mutations in melanoma cells increased cell motility and tumor formation in vivo. This was due in part to aberrant localization of truncated proteins to the mitochondria, regulating genes involved in cell survival, including CypD (PMID: 27759562). The TP53 S261Vfs*84 is a truncating mutation in a tumor suppressor gene, and therefore is likely pathogenic. There are no FDA-approved or NCCN-compendium listed treatments specifically for patients with TP53 S261Vfs*84 mutant gastrointestinal stromal tumors.

*VAF: Variant Allele Frequency

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7. Clinical Trials to consider

PDGFRA associated clinical trials

NCT02029001	PHASE2
Title	Adapting Treatment to the Tumor Molecular Alterations for Patients With Advanced Solid Tumors: MyOwnSpecificTreatment
Treatment	Nilotinib (400 mg BID) | Everolimus (10 mg QD) | Sorafenib (400 mg BID) | Lapatinib (1500 mg QD) | Pazopanib (800 mg QD) | Olaparib (300 mg BID) | Durvalumab + Tremelimumab
Location	France

NCT04771520	PHASE2
Title	Avapritinib for the Treatment of CKIT or PDGFRA Mutation-Positive Locally Advanced or Metastatic Malignant Solid Tumors
Treatment	Avapritinib
Location	United States

NCT07559864	PHASE2
Title	Comparing Regorafenib Combined With Envafolimab to Physician's Choice in Patients With Metastatic Gastrointestinal Stromal Tumors Harboring KIT Exon 17 Mutations Refractory to Standard Treatment
Treatment	physician-selected treatment | Regorafenib + Envafolimab
Location	China

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

NCT06630234	PHASE1 | PHASE2

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Name	-	Report No	26xxxxxxGR

Title	A Master Protocol to Evaluate DCC-3009 in Gastrointestinal Stromal Tumor (GIST)
Treatment	DCC-3009
Location	United States

Press here for a live search of clinical trials for PDGFRA

TP53 associated clinical trials

NCT06739395	**PHASE2**
Title	Precision Medicine Trial Based on Molecular Matching Therapy for Patients With Standard Treatment Exhaustion
Treatment	Olaparib tablet | Temozolomide capsule | Anlotinib | Trametinib tablet | Dabrafenib | Vebreltinib Enteric Capsules | Alpelisib Pill | Sacituzumab Govitecan-Hziy 180 MG | Lenvatinib Capsules | Pazopanib Pill | Palbociclib Pill | Chidamide | PD-1/PD-L1/PD-1&CTLA4 inhibitor | Target Gene
Location	China

NCT06329206	**PHASE1**
Title	A Phase Ia/Ib Study of GH2616 Tablet in Subjects With Advanced Solid Tumors
Treatment	GH2616 Tablets
Location	China

Press here for a live search of clinical trials for TP53

RB1 associated clinical trials

NCT06577987	**PHASE1**
Title	Safety/Efficacy Study of CID-078 in Patients With Advanced Solid Tumor Malignancies
Treatment	CID-078 Monotherapy
Location	United States

Press here for a live search of clinical trials for RB1

KIT associated clinical trials

NCT02029001	**PHASE2**

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Name	-	Report No	26xxxxxxGR

Title	Adapting Treatment to the Tumor Molecular Alterations for Patients With Advanced Solid Tumors: MyOwnSpecificTreatment
Treatment	Nilotinib (400 mg BID) | Everolimus (10 mg QD) | Sorafenib (400 mg BID) | Lapatinib (1500 mg QD) | Pazopanib (800 mg QD) | Olaparib (300 mg BID) | Durvalumab + Tremelimumab
Location	France

NCT05009927	**PHASE2**
Title	Efficiency of Imatinib Treatment After 10 Years of Treatment in Patients With Gastrointestinal Stromal Tumours (GIST) (Gist-Ten)
Treatment	Imatinib tablets
Location	France

NCT05159245	**PHASE2**
Title	The Finnish National Study to Facilitate Patient Access to Targeted Anti-cancer Drugs
Treatment	Alectinib | Cobimetinib | Vismodegib | Trastuzumab+Pertuzumab | Entrectinib | Atezolizumab | Vemurafenib | Regorafenib | Apalutamide | Abemaciclib | Tepotinib | Dabrafenib | Trametinib | Dabrafenib+Trametinib | Pemigatinib
Location	Finland

NCT04771520	**PHASE2**
Title	Avapritinib for the Treatment of CKIT or PDGFRA Mutation-Positive Locally Advanced or Metastatic Malignant Solid Tumors
Treatment	Avapritinib
Location	United States

NCT06630234	**PHASE1 | PHASE2**
Title	A Master Protocol to Evaluate DCC-3009 in Gastrointestinal Stromal Tumor (GIST)
Treatment	DCC-3009
Location	United States

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Name	-	Report No	26xxxxxxGR

NCT05366816	PHASE2
Title	ctDNA-Guided Sunitinib And Regorafenib Therapy for GIST
Treatment	Sunitinib | Regorafenib
Location	United States

NCT07171203	PHASE1
Title	Preoperative Imatinib and Fampridine in KIT Mutant Gastrointestinal Stromal Tumor
Treatment	Imatinib | Fampridine
Location	United States

NCT06805825	PHASE1
Title	A Study of the c-Kit Specific Antibody-Drug Conjugate NN3201 for Advanced and/or Metastatic Solid Tumors Known to Express c-Kit
Treatment	NN3201
Location	United States

Press here for a live search of clinical trials for KIT

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8. Appendix

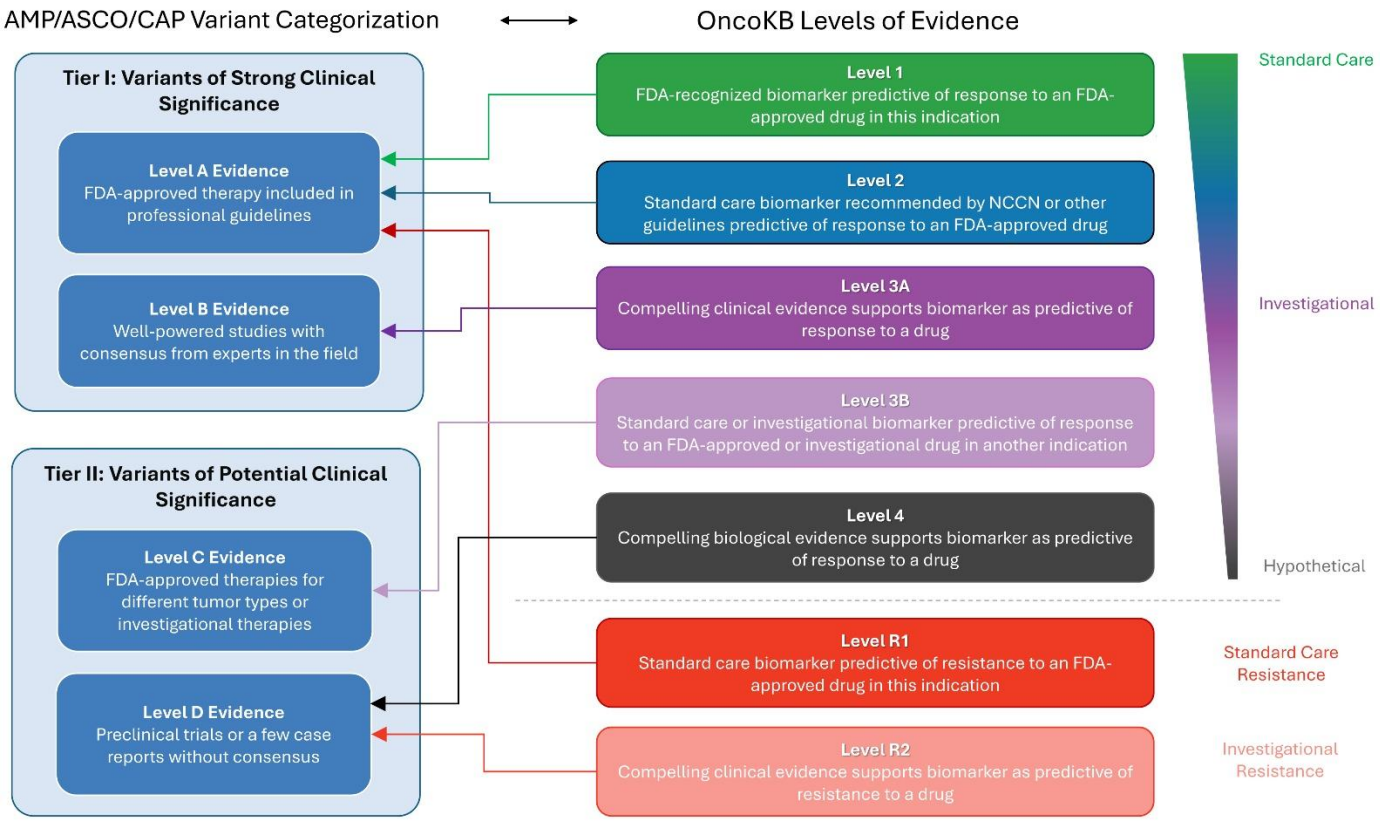

Figure 1. Joint consensus recommendation of AMP, ACMG, ASCO and CAP for reporting genetic variants in cancer. [1-2]

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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)	
PDGFRA	Exon 18 c.2525A>T (p.D842V) VAF: 0.3%	Avapritinib Dasatinib Regorafenib Ripretinib Sunitinib	-	-	-	Imatinib
KIT	Exon 11 c.1676_1684del (p.V559_E561del) VAF: 0.4%	Imatinib Sunitinib Regorafenib Ripretinib	-	-	-	-
RB1	Exon 8 c.763C>T (p.R255*) VAF: 2.1%	-	-	-	-	-
RB1	Exon 23 c.2342del (p.P781Qfs*29) VAF: 0.5%	-	-	-	-	-
TP53	Exon 7 c.780del (p.S261Vfs*84) VAF: 0.6%	-	-	-	-	-

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