# Com|Pl|i|t DX

| 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** | Melanoma |

**Com.Pl.i.t. Dx (21 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) | |
| ***BRAF*** | Exon 15<br>p.V600E<br>VAF: 21.6% | Dabrafenib<br>Dabrafenib+Trametinib<br>Encorafenib+Binimetinib<br>Trametinib<br>Vemurafenib<br>Vemurafenib+Atezolizumab+Cobimetinib<br>Vemurafenib+Cobimetinib | - | Encorafenib+Cetuximab<br>Encorafenib+Cetuximab+FOLFOX Regimen<br>Encorafenib+Panitumumab<br>Selumetinib<br>Tovorafenib | - |
| ***PTEN*** | Exon 8<br>p.S294Kfs*9<br>VAF: 23.6% | - | - | Capivasertib+Fulvestrant | - |
| ***TERT*** | c.-146C>T<br>VAF: 21.6% | - | - | - | - |
| **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

**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 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

**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.



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5. The detection method cannot between somatic mutations and germline mutations effectively without control sample analysis.
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.



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## 3. Quality Control Results

| Quality Control Index | Result | Criterion |
|---|---|---|
| Average effective sequencing depth[1] | **950** | ≥ 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 | **p.V600E (21.6%)** | *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 | **Detected** | **May decrease the benefit rate of PD-1/PD-L1 inhibitors** |



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## 6. Interpretations for targeted therapies

| Genetic Variation: | *BRAF*: c.1799T>A (p.V600E) | VAF*: 21.6% | OncoKB |
|---|---|---|---|

**Treatment Information**

The RAF-targeted inhibitors encorafenib, dabrafenib and vemurafenib alone or in combination with the MEK-targeted inhibitors binimetinib, trametinib and cobimetinib, respectively, are FDA-approved for the treatment of patients with BRAF V600E/K 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**

BRAF V600E is the most commonly reported BRAF alteration. V600E occurs within the BRAF kinase domain (amino acids 457-717; UniProt). This alteration gains 200- to 500-fold activity over the wild-type BRAF, constitutively activates MEK/ERK, resulting in up-regulation of cyclin D1 in the absence of extracellular signal, and induces cellular transformation (PMID: 21388974, 29533785). A genomic alteration resulting in this amino acid change is recorded within dbSNP (rs113488022, rs121913377) (Mar, 2018). The clinical significance of this alteration in ClinVar is Pathogenic, drug response, other, Likely pathogenic (Apr, 2021).

**Dabrafenib** DrugBank

Dabrafenib is an orally bioavailable RAF inhibitor that is FDA-approved for use in patients with BRAF V600E- and V600K-mutant metastatic melanoma. FDA approval is based on the randomized Phase III trial in which dabrafenib (150 mg orally twice daily) was compared with dacarbazine (1000 mg/m2 intravenously every three weeks) in 250 patients with BRAF V600E-mutated metastatic melanoma. Dabrafenib was associated with improved progression-free survival (median 5.1 months vs. 2.7 months with dacarbazine; hazard ratio = 0.30, p<0.0001) (PMID: 22735384). Dabrafenib may also be effective against brain metastases, as demonstrated by a Phase II trial in which approximately 40% of previously untreated and 30% of previously treated patients experienced an overall intracranial response and a separate trial in which nine out of ten patients had a reduction in the size of their brain metastases (PMID: 23051966, 22608338).

**Dabrafenib+Trametinib** DrugBank

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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 2 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). In the five-year update of the Phase III COLUMBUS trial, the progression-free survival and overall survival were 23% and 35% respectively in the encorafenib plus binimetinib group (n=192) versus 10% and 21% respectively in the single agent vemurafenib group (n=191), and the median duration of response and disease control rate were 18.6 months and 92.2% in the encorafenib plus binimetinib group versus 12.3 months and 81.2% in the single-agent vemurafenib group (PMID: 35862871).

### Trametinib

DrugBank

Trametinib is an oral small molecule inhibitor of MEK1/2 that is FDA-approved alone or with dabrafenib for the treatment of patients with metastatic melanoma harboring a V600E or V600K BRAF mutation. In an open-label, randomized Phase III trial, patients with BRAF V600E/K-mutated unresectable, metastatic melanoma received oral trametinib (2 mg once daily) or an intravenous regimen of either dacarbazine (1000 mg/m2) or paclitaxel (175 mg/m2) every three weeks. Trametinib demonstrated improved progression-free survival (HR for disease progression or death = 0.45) and six-month overall survival (81% vs. 67%; death HR = 0.54; p=0.01) (PMID: 22663011). However, like other MEK inhibitors, the benefit of trametinib is limited by adverse reactions, most notably grade three or four rash and diarrhea (PMID: 22663011). Trametinib is not typically used as monotherapy for patients with BRAF V600K melanoma given its

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lower response rate compared to BRAF inhibitors and combined BRAF and MEK inhibitors. Patients previously treated with a RAF inhibitor appear to be less likely than untreated patients to respond to trametinib treatment (PMID: 22663011), and FDA guidelines state that trametinib as a monotherapy is not indicated for these patients. Dabrafenib and trametinib are FDA-approved as a combination therapy, which has superior clinical outcomes compared to dabrafenib or trametinib monotherapy (PMID: 25399551, 25265492). Additionally, patients with melanoma treated with dabrafenib and trametinib in both the neoadjuvant and adjuvant settings had improved survival over patients given standard of care (PMID: 29361468).

### Vemurafenib

DrugBank

Vemurafenib is an orally available kinse inhibitor of V600-mutant BRAF that is FDA-approved for treatment of patients with unresectable or metastatic melanoma with the BRAF V600E mutation. Vemurafenib has been shown to have nearly equivalent activity against melanomas with BRAF V600E and V600K mutations (PMID: 24508103). In a randomized Phase III trial comparing vemurafenib (960 mg orally twice daily) with dacarbazine (1000 mg/m2 i.v. every 3 weeks) for treatment-naive, metastatic, BRAF V600E-mutant melanoma, vemurafenib was associated with better overall survival (median survival 13.6 months vs. 9.7 months; hazard ratio 0.70, p=.0008) and longer median progression-free survival (6.9 months vs. 1.6 months) (PMID: 24508103). Final overall survival data from the BRIM-3 study showed that the survival advantage of vemurafenib over dacarbazine persisted through the 4-year landmark, with survival rates for vemurafenib and dacarbazine at the 4-year landmark being 17.0% and 15.6%, respectively (PMID: 28961848). However, a trial evaluating clinical outcomes in patients with melanoma treated with either combination therapy of dabrafenib and trametinib compared to those treated with vemurafenib monotherapy demonstrated improved survival outcomes in the combination-therapy group compared to the vemurafenib group (PMID: 25399551).

### 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 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).

### Vemurafenib+Cobimetinib

DrugBank

The RAF inhibitor vemurafenib in combination with the MEK inhibitor cobimetinib 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 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



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group versus 38.0% in the monotherapy group (PMID: 27480103), and five-year followup of the BRIM7 study showed a median overall survival of 31.8 months and a five-year survival rate of 39.2% (PMID: 31732523).

### Encorafenib+Cetuximab

DrugBank

Encorafenib, a small molecule RAF-targeted inhibitor, and cetuximab, an anti-EGFR antibody, are FDA-approved in combination for the treatment of adult patients with metastatic CRC with a BRAF V600E mutation, as detected by an FDA-approved test, after prior therapy. BRAF V600E mutations for treatment with encorafenib plus cetuximab were detected by the FoundationOne Liquid CDx, the MI Cancer Seek assay or the therascreen BRAF V600E RGQ PCR Kit. FDA approval was based on results of the Phase III BEACON (NCT02928224) study of encorafenib plus cetuximab (n=220) versus triplet treatment (including a MEK1/2 inhibitor) versus chemotherapy (n=221) in 665 patients with BRAF V600E-mutant colorectal cancer.

In the Phase III BEACON (NCT02928224) trial, the overall response rate (complete or partial response) was 20% (95% CI=13-29) in the doublet arm versus 2% (95% CI=<1%-7%) in the chemotherapy arm (n=221), with median overall survival of 8.4 months in the doublet arm (95% CI= 7.5-11.0) and 5.4 months in the chemotherapy arm (95% CI= 4.8-6.6) (PMID: 31566309).

### Encorafenib+Cetuximab+FOLFOX Regimen

DrugBank

Encorafenib, a small molecule RAF-targeted inhibitor, and cetuximab, an anti-EGFR antibody, are FDA-approved in combination with FOLFOX6 for the treatment of patients with metastatic colorectal cancer (mCRC) with a BRAF V600E mutation, as detected by an FDA-approved test. BRAF V600E mutations were detected by the therascreen BRAF V600E RGQ PCR Kit. FDA approval was based on the results of the Phase III BREAKWATER (NCT04607421) trial of encorafenib plus cetuximab versus encorafenib plus cetuximab with FOLFOX6 versus standard-of-care in 479 patients with BRAF V600E-mutant mCRC.

In the Phase III BREAKWATER (NCT04607421) trial, the encorafenib plus cetuximab with FOLFOX6 cohort (n=110) demonstrated an overall response rate (ORR) of 60.9% (95% CI=51.6-69.5), with a 2.7% (n=3) complete response (CR) rate, 58.2% (n=64) partial response (PR) rate and 28.2% (n=31) stable disease (SD) rate, and a median duration of response (DOR) of 13.9 months (95% CI=8.5-NE) (PMID: 39863775). The standard-of-care cohort (n=110) demonstrated an ORR of 40.0% (95% CI=31.3-49.3) (odds ratio=2.443 [95% CI=1.403-4.253; 99.8% CI=1.019-5.855]; p=0.0008), with a 1.8% (n=2) CR rate, 38.2% (n=42) PR rate and 30.9% (n=34) SD rate, and a median DOR of 11.1 months (95% CI=6.7-12.7) (PMID: 39863775).

### Encorafenib+Panitumumab

DrugBank

Encorafenib, a small molecule inhibitor of RAF kinase, and panitumumab, an antibody that targets EGFR, are NCCN-compendium listed in combination as level 2A therapy for patients with BRAF V600E-positive colorectal cancer. In the Phase III BEACON study of encorafenib + cetuximab, another EGFR antibody, versus triplet treatment (including a MEK1/2 inhibitor) versus chemotherapy in 665 patients with BRAF V600E-mutant colorectal cancer, the overall response rate (complete or partial response) was 20% (95% CI= 13-29) in the doublet arm (n=220) versus 2% (95% CI= <1%-7%) in the chemotherapy arm (n=221), with median overall survival of 8.4

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months in the doublet arm (95% CI= 7.5-11.0) and 5.4 months in the control arm (95% CI= 4.8-6.6) (PMID: 31566309). Panitumumab has also been used in doublet and triplet combination therapy, with a response rate in one study of 26% (PMID: 29431699).

**Selumetinib** DrugBank

Selumetinib is a small molecule tyrosine kinase inhibitor of MEK1/2. In stratum one of the phase II PBTC study of selumetinib in 25 patients with pilocytic astrocytoma harboring a KIAA1549–BRAF fusion or BRAF V600E mutation, seven of eighteen patients with a KIAA1549–BRAF fusion had a partial response to treatment (PMID: 31151904). Additionally, the two year progression-free survival rate for the BRAF study population (n=25) was 70% (95% CI = 47–85) (PMID: 31151904).

**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).

| Genetic Variation: | ***PTEN*: c.881_893del (p.S294Kfs*9)** | **VAF*: 23.6%** | OncoKB |
|---|---|---|---|

**Treatment Information**

While the pan-AKT kinase inhibitor capivasertib in combination with the selective estrogen receptor degrader (SERD) fulvestrant is FDA-approved for the treatment of patients with PTEN-mutant ER+/HER2- metastatic breast cancer, the clinical utility of this combination in patients with PTEN S294Kfs*9 mutant melanoma is unknown. Laboratory data suggest that cancer cells with loss-of-function PTEN alterations may be sensitive to beta-isoform selective PI3K-targeted inhibitors, such as the investigational agents GSK2636771 and AZD8186.

**Gene information**

PTEN is a tumor suppressor that is one of the most frequently mutated genes in human cancer (PMID: 9072974, 9090379, 22473468). PTEN has several physiological functions, most notably operating as a phosphatase that converts phosphatidylinositol (3,4,5)-triphosphate (PIP3) to phosphatidylinositol (4,5)-diphosphate (PIP2) at the cell membrane (PMID: 18767981). Impairment of PTEN function through multiple mechanisms, including through non-synonymous mutations, results in PIP3 accumulation and constitutive activation of catabolic downstream AKT/mTOR signaling. Therefore, PTEN inactivation promotes cell growth, proliferation and survival (PMID: 12040186). Additionally, nuclear PTEN is thought to regulate RAD51 expression, and in this way is also associated with homologous recombination and repair of DNA strand breaks (PMID: 17218262, 23888040). Thus, loss of PTEN may also lead to



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

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greater genomic instability and provide a setting for the accumulation of other deleterious mutations. PTEN is frequently mutated in many types of human cancers (PMID: 15254063). Germline loss-of-function PTEN mutations occur in approximately 80% of patients with the cancer predisposition syndrome Cowden disease, which is associated with high-penetrance breast and thyroid cancer (PMID: 9467011, 24136893, 21430697).

**Variant Information**

PTEN deletions result in the loss of the protein. These mutations have been found in various cancers including breast cancer, endometrial cancer and glioblastoma and as germline mutations in Cowden syndrome or Bannayan-Riley-Ruvalcaba syndrome (PMID: 28572459). Heterozygous deletion of PTEN in a genetically engineered mouse model caused increased DNA damage and the development of spontaneous tumor formation and evidence of dysplastic changes, while homozygous deletion of PTEN is incompatible with life (PMID: 17218262, 9697695). PTEN is altered in 6.26% of colorectal carcinoma patients with PTEN loss present in 1.44% of all colorectal carcinoma patients (mycancergenome.org). PTEN deletion is known to be pathogenic.

**Capivasertib+Fulvestrant** DrugBank

Capivasertib, an orally available, ATP-competitive pan-AKT inhibitor, is FDA-approved with fulvestrant for the treatment of patients with PTEN-mutant ER+/HER2- metastatic breast cancer. FDA approval was based on the results of the Phase III CAPItello-291 trial of capivasertib plus fulvestrant in adult patients with or without AKT pathway-altered (PIK3CA, AKT1 or PTEN) ER+/HER2- advanced breast cancer. Of the patients with only PIK3CA, AKT1 or PTEN-mutant tumors (n=289), the capivasertib plus fulvestrant group (n=155) demonstrated an objective response rate of 26% (95% CI=19, 34), with a 2.3% complete response rate and 23% partial response rate, and a median progression-free survival of 7.3 months (95% CI=5.5, 9.0) whereas the placebo plus fulvestrant group (n=134) demonstrated an objective response rate of 8% (95% CI=4, 14), with a 8% partial response rate, and a median progression-free survival of 3.1 months (95% CI=2.0, 3.7) (HR=0.50 [95% CI=0.38, 0.65]; P<0.0001) (PMID: 37256976). Of all patients, including patients with wildtype tumors and patients with PIK3CA, AKT1 or PTEN-mutant tumors (n=708), the capivasertib plus fulvestrant group (n=355) demonstrated a median progression-free survival of 7.2 months (95% CI=5.5, 7.4) whereas the placebo plus fulvestrant group (n=353) demonstrated a median progression-free survival of 3.6 months (95% CI=2.8, 3.7) (HR=0.60 [95% CI=0.51, 0.71]; P<0.001) (PMID: 37256976).

| Genetic Variation: | *TERT*: c.-146C>T | VAF*: 21.6% | OncoKB |
|---|---|---|---|

**Treatment Information**

There are no FDA-approved or NCCN-compendium listed treatments specifically for patients with TERT 5:g.1295250G>A mutant melanoma.

**Gene information**

The TERT gene encodes the catalytic subunit of telomerase, an enzyme that maintains telomere length and genomic integrity. TERT expression is low or absent in somatic cells; however, telomerase activity is upregulated in a vast majority of tumors and likely contributes to cancer cell immortality (PMID: 24657534, 9282118). Sequencing of the TERT promoter identified activating mutations in

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a number of cancer types including melanoma, hepatocellular carcinoma, urothelial carcinoma, medulloblastoma and glioma (PMID: 23348506, 23530248). Tumors with highly recurrent TERT promoter mutations tend to originate from tissues with lower rates of self-renewal (PMID: 23530248). TERT promoter mutations, C228T and C250T, account for the majority of the somatic TERT promoter alterations and occur 124 and 146 base pairs upstream of the ATG start codon of TERT, respectively. Both promoter mutations create binding motifs for erythroblast transformation-specific (ETS)/ T-cell factor (TCF) transcription factors and enhance telomerase activity (PMID: 23348503, 23348506, 26194807). In addition to promoter mutations, TERT, located on chromosome 5p, is amplified across many cancer types (PMID: 20164920).

**Variant Information**

The C250T mutation (c.-146C>T) in the TERT gene is a somatic mutation that has been observed in various tumors, particularly in gliomas and meningiomas. It is located 146 base pairs upstream of the TERT transcription start site and is often associated with better prognosis compared to the C228T mutation in glioblastoma patients. The C250T mutation has been linked to worse overall survival in metastatic cutaneous melanoma, indicating its prognostic significance. TERT promoter mutations, including C250T, are highly prevalent across various cancers, such as melanoma and urothelial carcinoma, and are associated with increased tumor mutational burden. This mutation is likely pathogenic. There are no FDA-approved or NCCN-compendium listed treatments specifically for patients with TERT c.-146C>T mutant non-small cell lung cancer.

*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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|---|---|
| **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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|---|---|
| **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

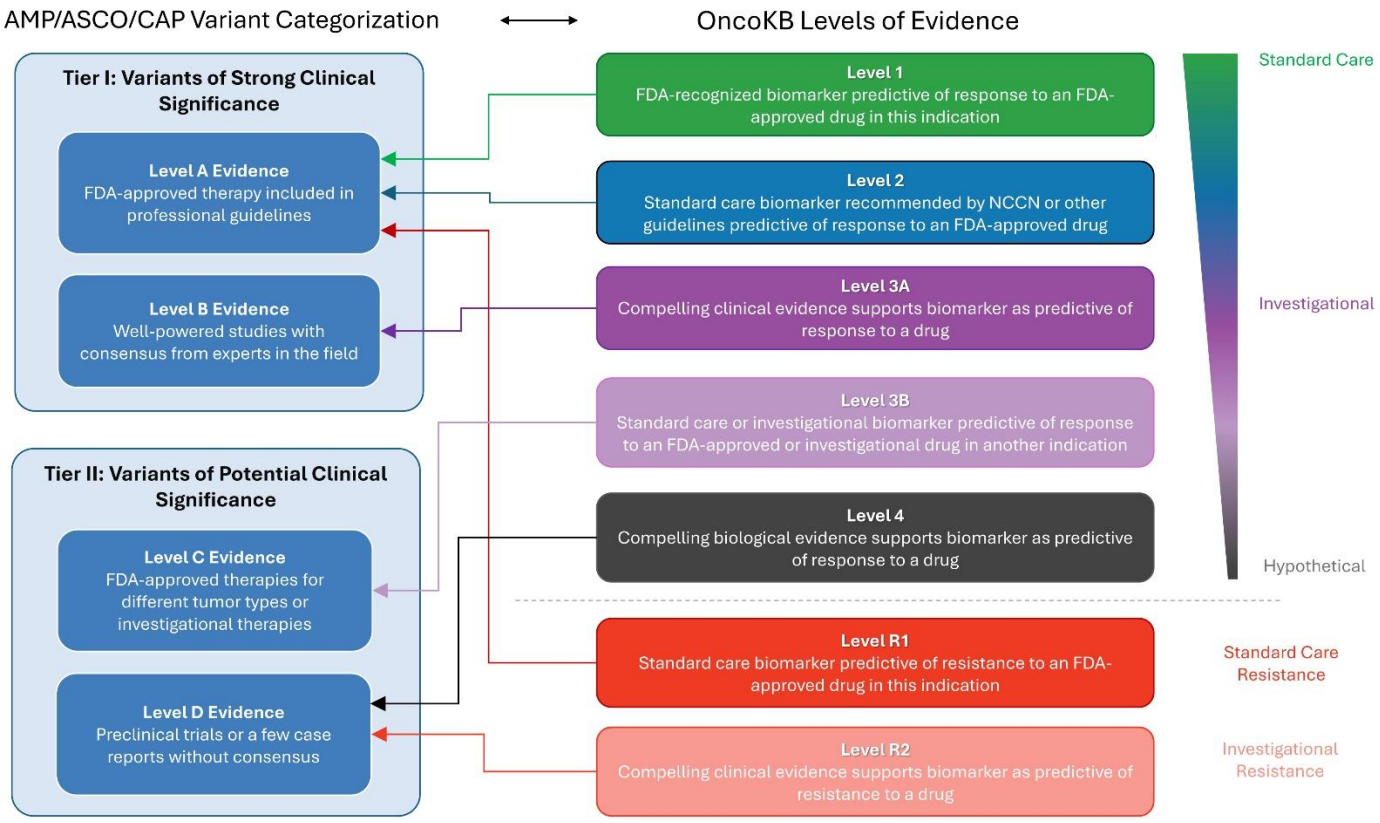


**Figure 1.** Joint consensus recommendation of AMP, ACMG, ASCO and CAP for reporting genetic variants in cancer. [1-2]

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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.1799T>A (p.V600E) VAF: 21.6% | Dabrafenib<br>Dabrafenib+Trametinib<br>Encorafenib+Binimetinib<br>Trametinib<br>Vemurafenib<br>Vemurafenib+Atezolizumab+Cobimetinib<br>Vemurafenib+Cobimetinib | - | Encorafenib+Cetuximab<br>Encorafenib+Cetuximab+FOLFOX Regimen<br>Encorafenib+Panitumumab<br>Selumetinib<br>Tovorafenib | - | - |
| **PTEN** | Exon 8 c.881_893del (p.S294Kfs*9) VAF: 23.6% | - | - | Capivasertib+Fulvestrant | GSK2636771<br>AZD8186 | - |
| **TERT** | Exon - c.-146C>T () VAF: 21.6% | - | - | - | - | - |



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