onkosight™ NGS

by ONCOLOGY genpath an OPKO Health Company

Clinical Significance of OnkoSight **Solid Tumor** Panel Genes

Rigorous review and selection of genes for OnkoSight NGS panels was performed to ensure that genes with actionable significance are included. Actionable significance is defined as those genes that have diagnostic, prognostic, and/or therapeutic predictive value, including those that may qualify patients for clinical trials. Panel components may be updated as new data is presented to ensure that OnkoSight provides optimal clinical benefit.

OnkoSight Solid Tumor Genes

Gene	Tumor Type(s)	Clinical Significance of Mutational Status	References (PubMedID)
AKT1	Breast cancer, Ovarian cancer, Colorectal cancer (CRC)	AKT1 mutations may predict response to AKT1/mTOR inhibitors.	17611497
ALK	NSCLC / Lung adenocarcinoma	Rearrangements of the ALK gene in NSCLC predict therapeutic response to ALK kinase inhibitors, including FDA-approved crizotinib and ceritinib, and resistance to EGFR inhibitors. Secondary ALK mutations and gene amplifications are associated with acquired resistance to crizotinib and variable response to second-generation ALK inhibitors.	NCCN-NSCLC 22277784 23729361 23744864
BRAF	Melanoma, CRC, Thyroid cancer, NSCLC / Lung adenocarcinoma, Ovarian cancer	Some mutations in BRAF predict response to RAF inhibitors in a variety of solid tumors. Therapies targeting BRAF V600-mutated melanoma (vemurafenib, dabrafenib, and trametinib) have been approved by the FDA. BRAF V600E is a negative prognostic marker in colorectal cancers, with resistance to anti-EGFR therapy in some clinical settings.	NCCN-Melanoma NCCN-NSCLC NCCN-Colon, Rectal 24388103 20008640 21739166
CTNNB1	CRC (MSI+), Endometrial cancer, Ovarian cancer, Melanoma, Desmoid tumors	Activating CTNNB1 mutations may predict response to WNT inhibitors.	24820952 10416591 23636398 18832571
DDR2	NSCLC / Squamous cell lung cancer	In squamous cell lung cancer, DDR2 mutations may predict response to dasatinib and other receptor tyrosine kinase inhibitors.	22328973 24828669 24296828
EGFR	NSCLC	Sensitizing EGFR mutations predict response to EGFR TKIs in NSCLC, including FDA-approved TKIs (erlotinib, gefitinib and afatinib), while other mutations predict resistance to first and second generation TKIs.	NCCN-NSCLC 15329413 15118073 15118125 15737014 15728811 23551194 18437168
EPHA2	NSCLC / Squamous cell lung cancer	Mutations in EPHA2 are more common in lung cancers with squamous cell histology. EPHA2 mutations have been associated with response to mTOR inhibitor (Rapamycin) in lung SCC.	20360610 23936763
ERBB2 (HER2)	NSCLC, Breast cancer	Mutations in ERBB2 (HER2) are emerging targets associated with response to anti-HER2 antibody (trastuzumab) or TKI (afatinib) therapy in NSCLC. In breast cancer, ERBB2 over-expression or gene amplification is associated with response to established anti-HER2 targeted therapies.	NCCN-NSCLC 23610105 22761469 16775247 18413839 23220880
ESR1	Breast cancer	Activating mutations in ESR1 are associated with acquired resistance to endocrine therapy in hormone-dependent metastatic breast cancer.	24398047
FGFR1	Breast cancer, NSCLC / Squamous cell lung cancer, Head and Neck cancer	Amplification of the FGFR1 gene predicts response to FGFR inhibition in several malignancies.	24265351 21367659
FGFR2	Breast cancer, Endometrial cancer, Bladder cancer, Gastric cancer	Alterations of FGFR2, including amplification, rearrangements and point mutations, predict response to FGFR inhibition in several malignancies.	24265351 21367659
FGFR3	Bladder cancer	Alterations of FGFR3, including amplification, rearrangements and point mutations, predict response to FGFR inhibition in several malignancies.	24265351 21367659

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Gene	Tumor Type(s)	Clinical Significance of Mutational Status	References (PubMedID)
GNA11 GNAQ	Uveal melanoma	Somatic mutations in either GNA11 or GNAQ genes are identified in the majority of uveal melanoma cases.	19078957 21083380 25304237
HRAS	Thyroid cancer, Cervical cancer, Urinary tract cancer, Head and Neck cancer, Skin cancer	RAS mutations have a high positive predictive value for malignancy in thyroid tumors, and aid in diagnosis of follicular thyroid lesions.	17384584 21739166
IDH1 IDH2	Glioma, Intrahepatic cholangiocarcinomas	IDH (IDH1 and IDH2) mutations are more prevalent in low grade diffuse gliomas and in secondary glioblastomas, they are associated with improved prognosis in low grade gliomas, and may predict response to therapy (IDH inhibitors, temolozomide).	20615753 20975057 23558169
KIT	Gastrointestinal Stromal Tumors (GIST), Melanoma	In GISTs, KIT mutations predict response to TKIs (imatinib, sunitinib), with secondary mutations associated with resistance. In metastatic melanoma cases, limited response to imatinib has been reported.	NCCN-Melanoma 18312355 21642685 21690468
KRAS	CRC, NSCLC / Lung adenocarcinoma, Thyroid cancer, Ovarian cancer	KRAS mutations in mCRC are predictive of lack of benefit to anti-EGFR therapeutic agents (cetuximab, panitumumab). In NSCLC, KRAS mutations are associated with primary EGFR TKI resistance.	NCCN-Colon, Rectal NCCN-NSCLC 24023839 19349489
MAP2K1	Melanoma, Lung adenocarcinoma	MAP2K1 mutations may confer resistance to BRAF and MEK inhibitors.	19915144
MET	Lung adenocarcinoma, Renal cancer	MET alterations in solid tumors, including amplification and mutations, may predict response to MET inhibitors. MET amplification has been associated with acquired resistance to anti-EGFR TKIs in lung cancer.	NCCN-NSCLC 24959084 18093943 17463250
MTOR	Endometrial cancer, Lung adenocarcinoma, Renal cancer	Activating mTOR mutations may predict response to mTOR inhibitors, while secondary mutations may confer acquired resistance.	24631838 25295501
NOTCH1	NSCLC, Head and neck SCC	NOTCH1 mutations may be associated with poor prognosis in a subset of NSCLC. NOTCH1 may contribute to TKI resistance in NSCLC.	25561229 23916913 20007775 25388163
NRAS	CRC, Thyroid cancer, Liver cancer	NRAS mutations in mCRC are predictive of lack of benefit to anti-EGFR therapeutic agents (cetuximab, panitumumab), and may predict response to MEK inhibitors in metastatic melanoma.	NCCN-Colon, Rectal 24806288 21739166 24820091 24419498
PDGFRA	Gastrointestinal Stromal Tumors (GIST)	PDGFRA mutations predict response to TKIs (imatinib, sunitinib).	18312355 15928335
PIK3CA	Breast cancer, Endometrial cancer, Urinary tract cancer, CRC	Mutations in PIK3CA may predict response to PI3K/AKT/mTOR inhibitors.	24333502 20085938
PTEN	Breast cancer, Endometrial cancer, Glioblastoma, CRC, Renal cancer	Mutations in PTEN may predict response to PI3K/AKT/mTOR inhibitors.	20085938 21430697
RAC1	Melanoma	RAC1 mutations have been reported to confer resistance to RAF inhibition in melanoma patients.	25956119
RET	Thyroid cancer, NSCLC	RET abnormalities, including rearrangements and mutations, predict response to multi-kinase inhibitors. RET point mutations have been associated with adverse prognosis in sporadic medullary thyroid carcinomas.	18073307 18437172 24561444
ROS1	NSCLC	ROS1 translocations predict therapeutic response to crizotinib in NSCLC, while secondary point mutations have been associated with acquired resistance.	NCCN-NSCLC 25264305 23724914
TP53	Most human cancers	Somatic mutations in the TP53 gene are found in the majority of human cancers, and are generally associated with adverse prognostic characteristics in a number of tumor types.	24651012 21045690

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481 Edward H Ross Drive Elmwood Park, NJ 07407 | 1 800 627 1479 tel | 1 201 345 7166 fax | www.genpath.com

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ONKOSIGHT TUMOR SEQUENCING FOR MYELOPROLIFERATIVE NEOPLASMS (MPNS)

2016 Revised World Health Organization Classification of Non-CML MPNs

In the spring of 2016, the World Health Organization (WHO) revised their classifications of myeloid neoplasms and acute leukemia. The previous update to these classifications was made in 2008. Between 2008 and 2016, there were **many advances in the identification of genetic biomarkers** important in the diagnosis and prognosis of myeloid neoplasms that warranted a classification revision. Our enhanced knowledge of genetic biomarkers was largely driven by the widespread availability of next-generation sequencing (NGS). As such, the NGS technology is well-suited to detect these relevant biomarkers in clinical specimens.

GenPath's next-generation sequencing OnkoSight MPN Panels cover the molecular testing needs outlined in the 2016 Revised WHO classification of myeloid neoplasms.

Biomarkers Included in the 2016 WHO Non-CML MPN Classifications[1,2,3]

(Full diagnostic information is not listed below. Morphologic distinction is still required for diagnosis and prognosis. The below information only addresses molecular characteristics of the diseases.)

Polycythemia vera (PV)	• Presence of *JAK2* V617F or *JAK2* exon 12 mutation (>95%)
Essential thrombocythemia (ET)	• Presence of *JAK2* (~60%), *MPL* (4%), or *CALR* (15-32%) mutation
Primary myelofibrosis (PMF)	• Presence of *JAK2* (~60%), *MPL* (~8%), or *CALR* (25-35%) mutation
Prefibrotic primary myelofibrosis (prePMF)	• Presence of *JAK2, MPL,* or *CALR* mutation
Chronic neutrophilic leukemia (CNL)	• Presence of *CSF3R* T618I or other activating *CSF3R* mutation

Prognosis and Other Relevant Biomarkers in Non-CML MPNs[2,3,4]

Non-CML MPN Classification	Median survival varies based on the non-CML MPN diagnosis: • ET, 19.8 years • PV, 13.5 years • PMF, 5.9 years
Mutation Status	• Median survival in ET and PV are not impacted by *JAK2, MPL,* or *CALR* status (similar prognosis regardless of which gene is mutated or not mutated) • **PMF** prognosis is highly dependent on mutation status with ***CALR+*** patients demonstrating the longest median survival (15.9 years) and triple negative patients the shortest (2.3 years) • **PMF *CALR- ASXL1+*** patients also exhibit very poor prognosis (2.3 years median survival)
Triple Negative ET or PMF (i.e., *JAK2-, MPL-, CALR-*)	• Mutations in genes common to other myeloid neoplasms such as splicing genes, *SF3B1* and *SRSF2,* and chromatin mutation genes, *ASXL1* and *EZH2,* are often seen • **Poorest mutational prognostic category in PMF** (5-10% of PMF cases)
Number of Mutations	• Research is beginning to suggest that the **number of mutations** present may correlate with the MPN classification (e.g., patients with PMF may have more mutations than patients with ET) • Research also suggests that the number of mutations may correlate with prognosis, but additional studies are needed to solidfy these findings
***JAK2* V617F Allelic Frequency**	• **Highest allele burdens** are found in PMF and PV compared to ET • PMF is often >50%, ET is often <40% (values are not absolute and histomorphologic assessment is essential)

References

1. **Blood.** 2016 May 19;127(20):2391-405. doi: 10.1182/blood-2016-03-643544. Epub 2016 Apr 11.
2. **Br J Haematol.** 2016 Nov;175(3):419-426. doi: 10.1111/bjh.14269. Epub 2016 Jul 22.
3. **Blood.** 2014 Oct 16;124(16):2507-13; quiz 2615. doi: 10.1182/blood-2014-05-579136. Epub 2014 Jul 18.
4. **Leukemia.** 2014 Jul;28(7):1472-7. doi: 10.1038/leu.2014.3. Epub 2014 Jan 9.

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OnkoSight Tumor Sequencing MPN Panels

GenPath currently offers three OnkoSight Tumor Sequencing MPN panel options to meet the various diagnostic needs of our patients and providers. Each panel is performed with our next-generation sequencing technology to provide an enhanced limit of detection, greater exon / mutation coverage, a single report for all genes interrogated with matching clinical trials, and faster turnaround time. Key elements across all of our OnkoSight MPN panels include:

- When *JAK2* is sequenced, it always tests for both V617F and exon 12 mutations.
- The limit of detection for *JAK2* V617F is 0.5% allelic frequency and 4% across all other genes*.
- Turnaround time for OnkoSight panels is 5-10 days from receipt of the specimen at GenPath.
- Accepted specimens include 1-2 mL of bone marrow or 2-4 mL of peripheral blood.
- Appropriate diagnosis codes (ICD-10 codes required) and a pathology report, if available, must accompany specimens for accurate results interpretation.

OnkoSight *JAK2, MPL, CALR* Panel

- 3 genes sequenced: *JAK2, MPL, CALR*
- **Clinical Utility:** Diagnosis/classification of PV, ET, PMF, and prePMF. Prognostic information in *CALR+* PMF (favorable).
- Test Code J633-9

OnkoSight *JAK2, MPL, CALR* Panel, if negative reflex to OnkoSight MPN Panel

- 3 genes sequenced: *JAK2, MPL, CALR*, if negative, reflex to the remaining 14 genes on the OnkoSight MPN Panel (please see gene list below).
- One report is issued with comprehensive interpretive information.
- **Clinical Utility:** Diagnosis/classification of triple negative MPNs, such as chronic neutrophilic leukemia (CNL) which is often *CSF3R+*, or other triple negative MPNs which may be positive for *SF3B1, SRSF2, ASXL1* or *EZH2* mutations. Prognostic information if triple negative or *CALR- ASXL1+* PMF (poor).
- Test Code J632-1

OnkoSight MPN Panel

- 17 genes sequenced: *ABL1, ASXL1, CALR, CBL, CSF3R, DNMT3A, EZH2, IDH1, IDH2, KIT, JAK2, MPL, SETBP1, SF3B1, SRSF2, TP53, U2AF1*
- **Clinical Utility:** Diagnosis/classification of PV, ET, PMF, prePMF, and triple negative MPNs, as well as prognostic information.
- Test Code B818-6

JAK2 V617F, *JAK2* exon 12, *MPL* 515, and *CALR* are also offered as PCR assays. *JAK2* V617F by PCR has the benefit of a faster 3 day turnaround time. However, the genetic areas interrogated by these PCR assays are more limited compared to their NGS counterparts.

*GenPath reports out confirmed disease associated variants at a 4% allelic frequency. For all other variants, the allelic frequency for reporting is 5% or greater.

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