Solid Tumor Mutation Panel by Next Generation Sequencing

Indications for Ordering

- Assess for targeted variants that are useful for prognosis and/or treatment of individuals with solid tumor cancers, including melanoma, gastrointestinal stromal tumor (GIST), colorectal, bladder, and hepatocellular carcinomas, at initial diagnosis or in the presence of refractory disease
- If the clinical indication is lung cancer, additional molecular genetic testing may be considered for detection of gene rearrangements and/or c-MET exon 14-skipping alterations
- For evaluation of microsatellite instability, additional molecular testing should be considered

Test Description

- Genomic DNA isolated from microscopically-guided dissection of tumor tissue
- Enrichment for mutational hotspot regions of interest in 44 genes by hybridization
- Variant status determined by massively parallel sequencing (next generation sequencing)

Tests to Consider

Primary test

Solid Tumor Mutation Panel by Next Generation Sequencing 2007991

Related tests

Mutation detection – single genes

- *BRAF* Codon 600 Mutation Detection by Pyrosequencing 2002498
- *BRAF* V600E Mutation Detection in Circulating Cell-Free DNA by Digital Droplet PCR 2013921
- *EGFR* Mutation Detection by Pyrosequencing 2002440
- *KIT* Mutations, Melanoma 2002695
- *KRAS* Mutation Detection 0040248
- *NRAS* Mutation Detection by Pyrosequencing 2003123
- *EGFR* T790M Mutation Detection in Circulating Tumor DNA by Digital Droplet PCR 2012868

Mutation detection – multiple genes or reflex assays

- Microsatellite Instability (MSI), HNPCC/Lynch Syndrome, by PCR 0051740
- Gastrointestinal Stromal Tumor Mutation 2002674
- *IDH1* and *IDH2* Mutation Analysis, exon 4 2006444
- *KRAS* Mutation Detection with Reflex to *BRAF* Codon 600 Mutation Detection 2001932

Non-small cell lung cancer – genetic alterations by FISH

- *ALK* Gene Rearrangements by FISH, Lung 2006102
- *ROS1* by FISH 2008418
- *RET* Gene Rearrangements by FISH 2012654
- *MET* Gene Amplification by FISH 2013082

Immunohistochemical assays

- *ALK* (D5F3) with Interpretation by Immunohistochemistry 2007324
- *ALK* (D5F3) by Immunohistochemistry with Reflex to *ALK* Gene Rearrangements by FISH 2011431
- *ROS1* with Interpretation by Immunohistochemistry with Reflex to FISH if Equivocal or Positive 2008414
- Mismatch Repair by Immunohistochemistry 0049302

Disease Overview

Incidence

- All cancers in U.S. – 473/100,000
- Deaths from cancer – 179/100,000

Diagnostic issues

- Genetic targets contained in panel, including extended *RAS* targets, are relevant across the spectrum of solid tumors
- Identification of one or more variants may aid in diagnostic subclassification

Prognostic and Treatment issues

- Certain gene variants may have prognostic significance
- Certain gene variants may be sensitive or provide resistance to available targeted therapies

Genetics

Genes – *AKT1, ALK, APC, ATM, BRAF, CDH1, CDKN2A, CTNNB1, DDR2, EGFR, ERBB2, ERBB4, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, GNA11, GNAQ, GNAS, HRAS, IDH1, IDH2, KDR, KIT, KRAS, MAP2K1, MET, MTOR, NOTCH1, NRAS, NTRK1, PDGFRA, PIK3CA, PTEN, RB1, RET, ROS1, SMAD4, SMO, STK11, TERT* promoter, *TP53, VHL*

Inheritance – somatic mutations
Variants– a full list of the targeted regions for the above genes can be found in the Solid Panel Coordinates table below

Test Interpretation

Results

- Positive
  - Variants in one or more of the 44 genes was detected
  - Clinical relevance (diagnosis, prognosis, or therapy) will be correlated, if known
- Negative
  - No pathogenic variants were detected

Limitations

- Does not detect translocations, gene rearrangements, copy number alterations, microsatellite instability, or tumor mutational burden
- Variants below the limit of detection (LOD) of 5% variant allele frequency may not be detected
- 10 ng input DNA from extracted tissue sample is minimally required, but 50 ng input DNA is recommended for optimal results
- Large variants (>60 base pairs [bp]) may not be detected
- Variants in known pseudogenes, homologous genomic regions, and/or low mappability regions may not be detected (see the Solid Panel Coordinates table)
- Not intended to detect minimal residual disease
- Does not distinguish between somatic and germline variants

Analytical Sensitivity

Variant Class	No. Variants Tested	Positive Percent Agreement (PPA)	PPA, 95% Tolerance at 95% Reliability
SNV	177	99%	97.4-99.9%
MNVs	42	93%	82.2-98.0%
Small insertions and duplications[a]	42	100%	95.6-100.0%
Medium insertions and duplications[b]	10	100%	82.9-100.0%
Large insertions[c]	1	100%	22.9%-100.0%
Small deletions[a]	80	100%	97.6-100.0%
Medium deletions[b]	14	100%	71.2%-99.2%
Large deletions[d]	22	64%	42.9%-81.1%

[a] ≤21bp
[b] 22-60bp
[c] ≥61 bp and ≤64bp
[d] ≥61 bp and ≤13547bp

Solid Panel Coordinates

Gene	Accession No.	Targeted Exons
AKT1	NM_001014431.1	3, 4, 6
ALK	NM_004304.4	16-29
APC	NM_000038.5	16[a]
ATM	NM_000051.3	8, 9, 12, 17, 26, 34-36, 39, 50, 54-56, 59, 61, 63
BRAF	NM_004333.4	11[b], 14, 15
CDH1	NM_004360.4	3, 8, 9
CDKN2A	NM_000077.4	2[b]
CTNNB1	NM_001904.3	3
DDR2	NM_001014796.1	18
EGFR	NM_005228.4	18-21
ERBB2	NM_004448.3	8, 17-22
ERBB4	NM_005235.2	3, 4, 6-9, 15, 23
EZH2	NM_004456.4	16, 18[b]
FBXW7	NM_033632.3	5, 8-11
FGFR1	NM_023110.2	4, 7
FGFR2	NM_000141.4	7, 9, 12
FGFR3	NM_000142.4	7, 9, 14, 16, 18
GNA11	NM_002067.4	5
GNAQ	NM_002072.4	5[b]
GNAS	NM_000516.5	8, 9
HRAS	NM_005343.3	2-4
IDH1	NM_005896.3	4
IDH2	NM_002168.3	4
KDR	NM_002253.2	6, 7, 11, 19, 21, 26 ,27, 30
KIT	NM_000222.2	2, 9, 10, 11, 13, 14, 15, 17, 18
KRAS	NM_004985.4	2, 3, 4
MAP2K1	NM_002755.3	2[b], 3, 6, 7[b], 11[b]
MET	NM_001127500.2	2[c] , 11, 13
MTOR	NM_004958.3	27-58
NOTCH1	NM_017617.4	26, 27, 34[d]
NRAS	NM_002524.4	2-5
NTRK1	NM_002529.3	5-15, 17
PDGFRA	NM_006206.4	12, 14, 15, 18
PIK3CA	NM_006218.2	2, 5, 7, 8, 10[b], 14[b], 19, 21
PTEN	NM_000314.6	1[b], 2[b], 3, 4-9[b]
RB1	NM_000321.2	4, 6, 10, 11, 14, 17, 18, 20, 21, 22
RET	NM_020975.4	6, 7, 8, 10-13, 15, 16
ROS1	NM_002944.2	7, 31-36, 38, 40, 41

Gene	Accession No.	Targeted Exons
SMAD4	NM_005359.5	3-12
SMO	NM_005631.4	3, 5, 6, 9-11
STK11	NM_000455.4	1, 4, 5, 6, 8
TERT Promoter	(NM_198253.2.1)	None [e]
TP53	NM_000546.5	2-11
VHL	NM_000551.3	1-3

[a] c.2390-c.2979, c.3128-c.3497, c.3730-4932
[b] Exon known to contain known pseudogenes, homologous genomic regions, and/or low mappability regions
[c] c.374-743, c.815-c.1200+10
[d] c.7168-c.7657
[e] Only c.-124C>T, c.-146C>T, c.-57 A>C, c.-125_124delinsTT and c.-139_-138delinsTT hotspot promoter variants reported