TruSight™ Oncology 500 ctDNA

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601 Genome Way
Huntsville, AL
35806

HUDSONALPHA DISCOVERY
SEQUENCING + BIOINFORMATICS™

STUDY	DISEASE	PARTICIPANT	REPORT DATE	REPORT STATUS
haib20SL6158	Control sample	6158-SL-0056		Final

CLINICALLY RELEVANT RESULTS

Tier I - Strong Clinical Significance

No variants were reported for this classification tier.

Tier II - Potential Clinical Significance

VARIANT	CLINICAL IMPACT
EGFR Copy number gain in *EGFR* (3 copies) C	**May benefit from** — Lapatinib *in Chordoma or Chondroid chordoma* INTERPRETATION Lapatinib used as single-agent therapy for the treatment of EGFR-positive recurrent conventional or chondroid chordoma (useful in certain circumstances).
MET Copy number gain in *MET* (3 copies) C	**May benefit from** — Crizotinib or Capmatinib *in Squamous cell carcinoma of lung, Adenocarcinoma of lung, Non-small cell lung cancer, Large cell carcinoma of lung, or Non-small cell carcinoma* **Not likely to benefit from** — Erlotinib, Afatinib, Osimertinib, or Gefitinib *in Non-small cell lung cancer or Non-small cell carcinoma*

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VARIANT | CLINICAL IMPACT

INTERPRETATION

(1) Most patients with sensitizing EGFR mutations become resistant to erlotinib, gefitinib, or afatinib; PFS is about 9.7 to 13 months. (2) Acquired resistance can also be mediated by other molecular events, such as acquisition of ALK rearrangement, MET, or ERBB2 amplification. (3) To overcome resistance, EGFR must still be inhibited. In the case of MET amplification, new inhibitors must be added to the EGFR inhibitor; EGFR inhibition is still required to induce remission. Furthermore, data show that when cancers start to progress, which were once sensitive to EGFR inhibitors, discontinuation of the EGFR TKI can lead to a much more accelerated progression of the cancer. Thus, continuing EGFR TKIs is beneficial in many patients even after they develop resistance to EGFR TKIs.

CSF3R

p.S469Afs*22
c.1404delC

C

NM_156039.3
VAF % 5.5
DEPTH 2,632

INTERPRETATION

Emerging data suggest that rare aCML patients with CSF3R or JAK2 mutations may respond to ruxolitinib therapy in combination with hypomethylating agents due to their JAK-STAT pathway activation.

NRAS

p.Q61K
c.181C>A

C

NM_002524.4
VAF % 12.3
DEPTH 2,392

May benefit from

— Binimetinib *in Lentigo maligna, Superficial spreading melanoma, Nodular malignant melanoma of skin, Superficial spreading malignant melanoma of skin, Nodular melanoma, Acral lentiginous malignant melanoma of skin, Desmoplastic malignant melanoma, or Malignant melanoma of skin*

Not likely to benefit from

— Panitumumab or Cetuximab *in Malignant tumor of colon, Primary adenocarcinoma of colon, Adenocarcinoma of rectum, or Malignant tumor of rectum*

Unfavorable Prognosis in

— Myelodysplastic syndrome (clinical) or Myelodysplastic syndrome

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INTERPRETATION

(1) Binimetinib is useful in certain circumstances as a single agent for metastatic or unresectable NRAS mutated tumors that have progressed after prior immune checkpoint inhibitor therapy. (2) In patients who were previously untreated or had prior failure of immunotherapy, binimetinib was associated with a response rate of 15%, and demonstrated a modest improvement in PFS with no improvement in OS compared with single-agent dacarbazine. (3) Binimetinib has also been shown to provide improved response rates and PFS compared with DTIC (dacarbazine) in a phase 3 randomized trial in patients with unresectable stage IIIC or stage IV melanoma with NRAS Q61R/K/L mutations.

MLH1

p.S252*
c.755C>A

C

NM_000249.3
VAF % 18.5
DEPTH 2,418

May benefit from

— Nivolumab or Ipilimumab + Nivolumab *in Malignant tumor of colon or Malignant tumor of rectum*

— Ipilimumab + Nivolumab *in Primary adenocarcinoma of colon, Adenocarcinoma of small intestine, or Adenocarcinoma of rectum*

— Pembrolizumab *in Malignant tumor of colon, Osteosarcoma of bone, Invasive micropapillary carcinoma of breast, Primary malignant neoplasm of endometrium, Malignant fibrous histiocytoma, Endometrial carcinoma, Adenocarcinoma of prostate, Siewert type II adenocarcinoma of esophagogastric junction, Adenocarcinoma of cervix, Adenocarcinoma of pancreas, Ewing's sarcoma of bone, Primary adenocarcinoma of colon, Squamous cell carcinoma of vulva, Malignant tumor of fallopian tube, Malignant tumor of small intestine, Adenocarcinoma of rectum, Infiltrating lobular carcinoma of breast, Malignant tumor of rectum, Malignant tumor of ovary, Seminoma - category, Carcinosarcoma of uterus, Mixed ductal and lobular carcinoma of breast, Adenocarcinoma of small intestine, Malignant tumor of biliary tract, Malignant tumor of esophagus, Malignant tumor of adrenal gland, Adenocarcinoma of esophagus, Infiltrating carcinoma with ductal and lobular features, Germ cell tumor, nonseminomatous, Squamous cell carcinoma of esophagus, Hepatocellular carcinoma, Malignant tumor of pancreas, Malignant tumor of testis, Malignant epithelial tumor of ovary, Chordoma, Malignant tumor of breast, Infiltrating duct carcinoma of breast, Malignant tumor of unknown origin or ill-defined site, Malignant tumor of cervix, Small cell carcinoma of lung, Inflammatory carcinoma of breast, Primary malignant neoplasm of the peritoneum, Carcinoma of esophagus, Siewert type III adenocarcinoma of esophagogastric junction, Malignant tumor of gallbladder, Siewert type I adenocarcinoma of esophagogastric junction, Malignant*

VARIANT	CLINICAL IMPACT

tumor of unknown origin, Cholangiocarcinoma of biliary tract, Primary malignant clear cell tumor of ovary, Carcinoma of cervix, Carcinosarcoma of ovary, Chondroid chordoma, Malignant retroperitoneal tumor, Malignant tumor of stomach, Malignant tumor of prostate, Dedifferentiated chondrosarcoma, Carcinoma, undifferentiated, Endometrioid carcinoma, Chondrosarcoma of bone, Malignant tumor of penis, Adenosquamous carcinoma of cervix, Endometrioid carcinoma ovary, Adenocarcinoma of stomach, Mucinous carcinoma of breast, or Mesenchymal chondrosarcoma

— Nivolumab *in Carcinoma, undifferentiated, Endometrioid carcinoma, Carcinosarcoma of uterus, Primary adenocarcinoma of colon, Adenocarcinoma of small intestine, Adenocarcinoma of rectum, or Endometrial carcinoma*

Favorable Prognosis in

— Malignant tumor of colon, Primary adenocarcinoma of colon, or Malignant tumor of rectum

INTERPRETATION

(1) Pembrolizumab is recommended as a single agent therapy for recurrent or stage IV (M1) disease that is unresectable or metastatic and microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR), or tumor mutational burden-high (TMB-H) tumors (greater than or equal to 10 muts/mb) that have progressed following prior treatment and has no satisfactory alternative treatment options. (useful in certain circumstances) (2) Pembrolizumab is U.S FDA approved for the treatment of patients with unresectable or metastatic, microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options.

CTNNB1

p.S33Y
c.98C>A

C

NM_001098209.1
VAF % 31.1
DEPTH 3,071

Diagnostic of

— Medulloblastoma

INTERPRETATION

(1) Description: Medulloblastomas are WHO grade IV tumors that predominantly arise from the cerebellum in pediatric patients, but can also occur in adults. The WHO committee on CNS tumors now recommends subclassification of these tumors into four distinct groups: i) WNT-activated; ii) SHH-activated and TP53-mutant; iii)

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INTERPRETATION

SHH-activated and TP53-wildtype; and iv) non-WNT/non-SHH. (2) Detection: Virtually all WNT-driven medulloblastomas will contain mutations in either CTNNB1 or, less commonly, APC (the latter mutation may be germline if the patient has Turcot syndrome). WNT-driven tumors will also usually contain monosomy 6. Differentiating between WNT-activated, SHH-activated, and non-WNT/non-SHH tumors is best classified by expression arrays, DNA methylation arrays, or an immunohistochemistry panel composed of beta-catenin, GAB1, and YAP1. (3) Molecular profiling to identify clinically relevant subtypes is recommended to encourage opportunities for clinical trial.

GATA2

p.G200Vfs*18
c.599delG

C

NM_032638.4
VAF % 9.1
DEPTH 3,951

Unfavorable Prognosis in

— Myelodysplastic syndrome (clinical) or Myelodysplastic syndrome

INTERPRETATION

Genes frequently somatically mutated in MDS include GATA2. Nonsense or Frameshift or Splice site mutations in GATA2 are associated with a poor prognosis.

PIK3CA

p.E545K
c.1633G>A

C

NM_006218.2
VAF % 8.4
DEPTH 2,676

May benefit from

— Alpelisib *in Human epidermal growth factor 2 negative carcinoma of breast, Malignant tumor of breast, Infiltrating duct carcinoma of breast, Invasive micropapillary carcinoma of breast, Mixed ductal and lobular carcinoma of breast, Mucinous carcinoma of breast, Inflammatory carcinoma of breast, Hormone receptor positive malignant neoplasm of breast, Infiltrating carcinoma with ductal and lobular features, or Infiltrating lobular carcinoma of breast*

INTERPRETATION

(1) Alpelisib is recommended for treatment of recurrent or stage IV (M1) hormone receptor-positive, human epidermal growth factor receptor 2 (HER2)-negative disease with no visceral crisis in postmenopausal women or premenopausal women treated with ovarian ablation/suppression, as second-line therapy or beyond in combination with fulvestrant if PIK3CA activating mutation positive (preferred regimen). (2) Men with breast cancer should be treated similarly to postmenopausal

VARIANT	CLINICAL IMPACT

INTERPRETATION

women, except that use of an aromatase inhibitor is ineffective without concomitant suppression of testicular steroidogenesis. (3) If there is disease progression while on a CDK4/6 or PI3K inhibitor, there are limited data to support an additional line of therapy with another CDK4/6- or PIK3CA-containing regimen. If there is disease progression while on a everolimus-containing regimen, there are no data to support an additional line of therapy with another everolimus regimen.

PIK3CA

p.H1047R
c.3140A>G

C

NM_006218.2
VAF % 16.4
DEPTH 3,072

May benefit from

— Alpelisib *in Human epidermal growth factor 2 negative carcinoma of breast, Malignant tumor of breast, Infiltrating duct carcinoma of breast, Invasive micropapillary carcinoma of breast, Mixed ductal and lobular carcinoma of breast, Mucinous carcinoma of breast, Inflammatory carcinoma of breast, Hormone receptor positive malignant neoplasm of breast, Infiltrating carcinoma with ductal and lobular features, or Infiltrating lobular carcinoma of breast*

INTERPRETATION

(1) Alpelisib is recommended for treatment of recurrent or stage IV (M1) hormone receptor-positive, human epidermal growth factor receptor 2 (HER2)-negative disease with no visceral crisis in postmenopausal women or premenopausal women treated with ovarian ablation/suppression, as second-line therapy or beyond in combination with fulvestrant if PIK3CA activating mutation positive (preferred regimen). (2) Men with breast cancer should be treated similarly to postmenopausal women, except that use of an aromatase inhibitor is ineffective without concomitant suppression of testicular steroidogenesis. (3) If there is disease progression while on a CDK4/6 or PI3K inhibitor, there are limited data to support an additional line of therapy with another CDK4/6- or PIK3CA-containing regimen. If there is disease progression while on a everolimus-containing regimen, there are no data to support an additional line of therapy with another everolimus regimen.

KIT

p.D816V
c.2447A>T

May benefit from

— Midostaurin *in Chronic myelomonocytic leukemia or Myelodysplastic/ myeloproliferative disease*

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C NM_000222.2 VAF % 8.5 DEPTH 2,958	**Not likely to benefit from** — Imatinib *in Aggressive systemic mastocytosis* **Diagnostic of** — Gastrointestinal stromal tumor INTERPRETATION (1) Patients with CMML may also have systemic mastocytosis with an associated hematologic neoplasm (SM-AHN) and KIT816V mutation responsive to midostaurin. (2) Midostaurin may be used as a single agent for the treatment of Chronic myelomonocytic leukemia (CMML)-0, CMML-1, or CMML-2 in patients with CMML-associated systemic mastocytosis (SM-AHN) and KIT816V mutation.
TET2 p.L1329M c.3985C>A C NM_001127208.2 VAF % 25.8 DEPTH 1,856	**Unfavorable Prognosis in** — Myeloproliferative disorder or Myeloproliferative neoplasm INTERPRETATION (1) TET2 or TP53 mutations have also been associated with a worsened overall prognosis and an increased rate of leukemic transformation. (2) Mutations in several genes (ASXL1, TET2, TP53, SRSF2, and IDH1 or IDH2) and other chromosomal abnormalities (eg, aberrations in chromosomes 1q and 9p) have been associated with transformation to AML.
SDHA c.1794+119delA C NM_004168.2 VAF % 38.2 DEPTH 3,478	**Diagnostic of** — Paraganglioma, malignant or Gastrointestinal stromal tumor INTERPRETATION (1) Morphologic diagnosis based on microscopic examination of histologic sections remains the gold standard for sarcoma diagnosis. However, several ancillary techniques are useful in support of morphologic diagnosis, including IHC, classical cytogenetics, electron microscopy, and molecular genetic testing. Molecular genetic testing has emerged as an ancillary testing approach since many sarcoma types

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INTERPRETATION

harbor characteristic genetic aberrations. Recurrent genetic aberrations in sarcoma include, germline SDH subunit mutations in familial gastric GIST and paraganglioma associated with Carney-Stratakis syndrome. (2) Carney-Stratakis syndrome is an autosomal-dominant familial syndrome characterized by a predisposition to GISTs and paragangliomas. Germline loss-of-function mutations within the succinate dehydrogenase (SDH) gene subunits (SDHB, SDHC, and SDHD) have been identified in individuals with GISTs associated with Carney-Stratakis syndrome. In an analysis of 11 patients from 9 families presenting with GIST and paragangliomas associated with Carney-Stratakis syndrome, Pasini and colleagues identified germline mutations in SDHB, SDHC, or SDHD genes in 8 patients (from 7 untreated families) with GISTs.

EGFR

p.G719S
c.2155G>A

C

NM_005228.3
VAF % 23.4
DEPTH 3,850

May benefit from

— Afatinib + Cetuximab or Afatinib *in Squamous cell carcinoma of lung, Adenocarcinoma of lung, Non-small cell lung cancer, Large cell carcinoma of lung, or Non-small cell carcinoma*

— Bevacizumab-bvzr + Erlotinib, Bevacizumab + Erlotinib, or Bevacizumab-awwb + Erlotinib *in Nonsquamous nonsmall cell neoplasm of lung, Adenocarcinoma of lung, or Large cell carcinoma of lung*

— Erlotinib, Erlotinib + Ramucirumab, Osimertinib, Dacomitinib, or Gefitinib *in Squamous cell carcinoma of lung, Adenocarcinoma of lung, Non-small cell lung cancer, Epidermal growth factor receptor positive non-small cell lung cancer, Large cell carcinoma of lung, or Non-small cell carcinoma*

— Afatinib *in Epidermal growth factor receptor positive non-small cell lung cancer*

Not likely to benefit from

— Afatinib, Erlotinib, Dacomitinib, or Gefitinib *in Squamous cell carcinoma of lung, Adenocarcinoma of lung, Non-small cell lung cancer, Epidermal growth factor receptor positive non-small cell lung cancer, Large cell carcinoma of lung, or Non-small cell carcinoma*

— Osimertinib *in Non-small cell lung cancer or Non-small cell carcinoma*

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INTERPRETATION

(1) Most patients with sensitizing EGFR mutations become resistant to erlotinib, gefitinib, or afatinib; PFS is about 9.7 to 13 months. (2) Acquired resistance can also be mediated by other molecular events, such as acquisition of ALK rearrangement, MET, or ERBB2 amplification. (3) To overcome resistance, EGFR must still be inhibited. In the case of MET amplification, new inhibitors must be added to the EGFR inhibitor; EGFR inhibition is still required to induce remission. Furthermore, data show that when cancers start to progress, which were once sensitive to EGFR inhibitors, discontinuation of the EGFR TKI can lead to a much more accelerated progression of the cancer. Thus, continuing EGFR TKIs is beneficial in many patients even after they develop resistance to EGFR TKIs.

EGFR

p.E746_A750del
c.2235_2249del15

C

NM_005228.3
VAF % 1.3
DEPTH 4,866

May benefit from

— Afatinib + Cetuximab or Afatinib *in Squamous cell carcinoma of lung, Adenocarcinoma of lung, Non-small cell lung cancer, Large cell carcinoma of lung, or Non-small cell carcinoma*

— Bevacizumab-bvzr + Erlotinib, Bevacizumab + Erlotinib, or Bevacizumab-awwb + Erlotinib *in Nonsquamous nonsmall cell neoplasm of lung, Adenocarcinoma of lung, or Large cell carcinoma of lung*

— Erlotinib, Erlotinib + Ramucirumab, Osimertinib, Dacomitinib, or Gefitinib *in Squamous cell carcinoma of lung, Adenocarcinoma of lung, Non-small cell lung cancer, Epidermal growth factor receptor positive non-small cell lung cancer, Large cell carcinoma of lung, or Non-small cell carcinoma*

— Afatinib *in Epidermal growth factor receptor positive non-small cell lung cancer*

Not likely to benefit from

— Erlotinib, Afatinib, Dacomitinib, or Gefitinib *in Squamous cell carcinoma of lung, Adenocarcinoma of lung, Non-small cell lung cancer, Epidermal growth factor receptor positive non-small cell lung cancer, Large cell carcinoma of lung, or Non-small cell carcinoma*

— Osimertinib *in Non-small cell lung cancer or Non-small cell carcinoma*

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INTERPRETATION

(1) Most patients with sensitizing EGFR mutations become resistant to erlotinib, gefitinib, or afatinib; PFS is about 9.7 to 13 months. (2) Acquired resistance can also be mediated by other molecular events, such as acquisition of ALK rearrangement, MET, or ERBB2 amplification. (3) To overcome resistance, EGFR must still be inhibited. In the case of MET amplification, new inhibitors must be added to the EGFR inhibitor; EGFR inhibition is still required to induce remission. Furthermore, data show that when cancers start to progress, which were once sensitive to EGFR inhibitors, discontinuation of the EGFR TKI can lead to a much more accelerated progression of the cancer. Thus, continuing EGFR TKIs is beneficial in many patients even after they develop resistance to EGFR TKIs.

EGFR

p.T790M
c.2369C>T

C

NM_005228.3
VAF % 0.8
DEPTH 5,813

May benefit from

— Osimertinib *in Squamous cell carcinoma of lung, Adenocarcinoma of lung, Non-small cell lung cancer, Epidermal growth factor receptor positive non-small cell lung cancer, Large cell carcinoma of lung, or Non-small cell carcinoma*

— Afatinib + Cetuximab *in Squamous cell carcinoma of lung, Adenocarcinoma of lung, Non-small cell lung cancer, Large cell carcinoma of lung, or Non-small cell carcinoma*

Not likely to benefit from

— Afatinib, Erlotinib, Dacomitinib, or Gefitinib *in Squamous cell carcinoma of lung, Adenocarcinoma of lung, Non-small cell lung cancer, Epidermal growth factor receptor positive non-small cell lung cancer, Large cell carcinoma of lung, or Non-small cell carcinoma*

INTERPRETATION

(1) Most patients with sensitizing EGFR mutations become resistant to erlotinib, gefitinib, or afatinib; progression-free survival (PFS) is about 9.7 to 13 months. (2) The most common known mechanism is the acquisition of T790M (which is a secondary mutation in EGFR), which renders the kinase resistant to erlotinib, gefitinib, dacomitinib, or afatinib. Therefore, if patients are T790M positive, osimertinib is recommended (category 1) and erlotinib, gefitinib, dacomitinib, or afatinib are discontinued. (3) The presence of p.T790M can direct patients to third-generation EGFR TKI therapy. (4) Data show that when cancers start to progress,

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INTERPRETATION

which were once sensitive to EGFR inhibitors, discontinuation of the EGFR TKI can lead to a much more accelerated progression of the cancer. Thus, continuing EGFR TKIs is beneficial in many patients even after they develop resistance to EGFR TKIs.

EGFR

p.L858R
c.2573T>G

C

NM_005228.3
VAF % 2.3
DEPTH 5,355

May benefit from

— Bevacizumab-bvzr + Erlotinib, Bevacizumab + Erlotinib, or Bevacizumab-awwb + Erlotinib *in Nonsquamous nonsmall cell neoplasm of lung, Adenocarcinoma of lung, or Large cell carcinoma of lung*

— Erlotinib, Erlotinib + Ramucirumab, Osimertinib, Dacomitinib, or Gefitinib *in Squamous cell carcinoma of lung, Adenocarcinoma of lung, Non-small cell lung cancer, Epidermal growth factor receptor positive non-small cell lung cancer, Large cell carcinoma of lung, or Non-small cell carcinoma*

— Afatinib + Cetuximab or Afatinib *in Squamous cell carcinoma of lung, Adenocarcinoma of lung, Non-small cell lung cancer, Large cell carcinoma of lung, or Non-small cell carcinoma*

— Afatinib *in Epidermal growth factor receptor positive non-small cell lung cancer*

Not likely to benefit from

— Erlotinib, Afatinib, Dacomitinib, or Gefitinib *in Squamous cell carcinoma of lung, Adenocarcinoma of lung, Non-small cell lung cancer, Epidermal growth factor receptor positive non-small cell lung cancer, Large cell carcinoma of lung, or Non-small cell carcinoma*

— Osimertinib *in Non-small cell lung cancer or Non-small cell carcinoma*

INTERPRETATION

(1) Most patients with sensitizing EGFR mutations become resistant to erlotinib, gefitinib, or afatinib; PFS is about 9.7 to 13 months. (2) Acquired resistance can also be mediated by other molecular events, such as acquisition of ALK rearrangement, MET, or ERBB2 amplification. (3) To overcome resistance, EGFR must still be inhibited. In the case of MET amplification, new inhibitors must be added to the EGFR inhibitor; EGFR inhibition is still required to induce remission. Furthermore, data show that when cancers start to progress, which were once sensitive to EGFR inhibitors, discontinuation of the EGFR TKI can lead to a much more accelerated

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INTERPRETATION

progression of the cancer. Thus, continuing EGFR TKIs is beneficial in many patients even after they develop resistance to EGFR TKIs.

BRAF

p.V600E
c.1799T>A

C

NM_004333.4
VAF % 9.6
DEPTH 4,030

May benefit from

— Vemurafenib, Pembrolizumab, Ipilimumab + Talimogene laherparepvec, Ipilimumab + Nivolumab, Ipilimumab, Nivolumab, Dabrafenib, Cobimetinib + Vemurafenib, Atezolizumab + Cobimetinib + Vemurafenib, Dabrafenib + Trametinib, or Binimetinib + Encorafenib *in Lentigo maligna, Superficial spreading melanoma, Nodular malignant melanoma of skin, Superficial spreading malignant melanoma of skin, Nodular melanoma, Acral lentiginous malignant melanoma of skin, Desmoplastic malignant melanoma, or Malignant melanoma of skin*

— Cobimetinib + Vemurafenib or Dabrafenib + Trametinib *in Dysembryoplastic neuroepithelial tumor, Ganglioglioma, anaplastic, Pilocytic astrocytoma, Low grade glioma, or Pleomorphic xanthoastrocytoma*

— Bevacizumab-bvzr, Bevacizumab-awwb, Bevacizumab, or Atezolizumab *in Nonsquamous nonsmall cell neoplasm of lung, Adenocarcinoma of lung, or Large cell carcinoma of lung*

— Pembrolizumab or Ipilimumab + Nivolumab *in Squamous cell carcinoma of lung, Adenocarcinoma of lung, Non-small cell lung cancer, Large cell carcinoma of lung, or Non-small cell carcinoma*

— Encorafenib + Panitumumab or Cetuximab + Encorafenib *in Malignant tumor of colon, Primary adenocarcinoma of colon, Adenocarcinoma of rectum, or Malignant tumor of rectum*

— Dabrafenib + Trametinib *in Malignant melanoma, metastatic, Squamous cell carcinoma of lung, Adenocarcinoma of lung, Non-small cell lung cancer, Malignant melanoma, Large cell carcinoma of lung, Non-small cell carcinoma, Metastatic malignant melanoma, or Anaplastic thyroid carcinoma*

— Cobimetinib + Vemurafenib, Binimetinib + Encorafenib, Atezolizumab + Cobimetinib + Vemurafenib, or Trametinib *in Malignant melanoma, metastatic, Malignant melanoma, or Metastatic malignant melanoma*

— Vemurafenib *in Malignant melanoma, metastatic, Squamous cell carcinoma of lung, Hurthle cell carcinoma of thyroid, Non-small cell carcinoma, Hairy cell leukemia, Follicular thyroid carcinoma, Hairy cell leukemia (clinical), Adenocarcinoma of lung, Non-small cell lung cancer, Malignant melanoma, Erdheim-Chester disease, Large cell*

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carcinoma of lung, Papillary thyroid carcinoma, Metastatic malignant melanoma, or Polyostotic sclerosing histiocytosis

— Dabrafenib *in Malignant melanoma, metastatic, Follicular thyroid carcinoma, Adenocarcinoma of lung, Malignant melanoma, Non-small cell lung cancer, Hurthle cell carcinoma of thyroid, Non-small cell carcinoma, Papillary thyroid carcinoma, or Metastatic malignant melanoma*

Not likely to benefit from

— Panitumumab or Cetuximab *in Malignant tumor of colon, Primary adenocarcinoma of colon, Adenocarcinoma of rectum, or Malignant tumor of rectum*

Unfavorable Prognosis in

— Malignant tumor of colon, Primary adenocarcinoma of colon, Adenocarcinoma of rectum, Papillary thyroid carcinoma, or Malignant tumor of rectum

Diagnostic of

— Hairy cell leukemia or Hairy cell leukemia (clinical)

INTERPRETATION

(1) Recommendation: BRAF p.V600 (BRAF c. 1799 (p.V600) mutational analysis should be performed in colorectal cancer tissue in patients with colorectal carcinoma for prognostic stratification (Type: recommendation, Strength of Evidence: adequate/inadequate, balance of benefits and harms; Quality of Evidence: intermediate/low). (2) Patients with advanced CRC who possess a BRAF mutation have significantly poorer outcomes as measured by PFS and OS and have a decreased response rate to anti-EGFR therapy relative to those with nonmutated BRAF. Poorer OS was also demonstrated for those patients with earlier stage II and III CRC having a BRAF mutation; however, the poorer outcome appears to be primarily the result of decreased OS after relapse in these patients rather than a harbinger of an increased rate of relapse. Finally, while outcomes in advanced disease patients with BRAF mutations were poorer relative to nonmutation patients, the data were consistent with a modest beneficial impact from the use of anti-EGFR agents relative to those patients whose tumors contained a RAS mutation. In summary, patients with CRC that contains a BRAF mutation have a worse outcome relative to nonmutation patients. Selected patients for BRAF mutation testing include patients with metastatic disease, since these patients have particularly poor outcomes. It is important to know the BRAF c.1799 (p.V600) mutation status of a patient's CRC since standard therapy is not adequate for patients with metastatic disease and BRAF

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mutation.Data in support of molecular testing for BRAF c.1799 (p.V600) mutations in CRC continue to emerge from clinical trials. A recent publication of the PETACC-8 (Oxaliplatin, Fluorouracil, and Leucovorin With or Without Cetuximab in Patients With Resected Stage III Colon Cancer Randomized Phase III) trial reported that trials in the adjuvant setting should consider mismatch repair, BRAF, and KRAS status for stratification, since BRAF p.V600 and KRAS mutations were associated with shorter DFS and OS in patients with microsatellite-stable colon cancer but not in those with tumors with MSI. (3) The presence of deficient MMR or BRAF p.V600E mutation in proficient MMR CRCs has important prognostic significance. (4) BRAF mutations are consistently associated with poor outcomes in patients with metastatic CRC, including those who relapse after adjuvant therapy.

EZH2

p.G395Efs*29
c.1184delG

C

NM_004456.4
VAF % 22.4
DEPTH 4,819

Unfavorable Prognosis in

— Myelodysplastic syndrome (clinical), Myelodysplastic/myeloproliferative disease, or Myelodysplastic syndrome

INTERPRETATION

(1) Genes frequently somatically mutated in MDS include EZH2. Nonsense or Frameshift mutations in EZH2 are independently associated with a poor prognosis in MDS and MDS/MPN. (2) Mutations of TP53, EZH2, ETV6, RUNX1, and ASXL1 have been shown to predict decreased OS in multivariable models adjusted for IPSS or IPSS-R risk groups in several studies of distinct cohorts.

PTCH1

p.S1203Afs*52
c.3606delC

C

NM_000264.3
VAF % 28.6
DEPTH 3,713

May benefit from

— Vismodegib *in Medulloblastoma*

INTERPRETATION

(1) Vismodegib is a recommended treatment for recurrence as a single agent (useful in certain circumstances) in patients who have received prior chemotherapy and have mutations in the sonic hedgehog pathway. (2) SHH-pathway inhibitors that have been evaluated in phase II trials including adults with recurrent medulloblastoma include vismodegib and sonidegib. Patients in these trials with

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VARIANT	CLINICAL IMPACT
	INTERPRETATION SHH-activated disease were more likely to respond than patients with non-SHH disease.
ATM p.R1973Efs*17 c.5917delA C NM_000051.3 VAF % 11.2 DEPTH 1,436	**May benefit from** — Olaparib *in Malignant tumor of prostate or Adenocarcinoma of prostate* INTERPRETATION (1) Olaparib is useful in certain circumstances as a single-agent second-line or subsequent treatment for castration-resistant distant metastatic (M1) disease for patients who have been treated with androgen receptor-directed therapy and have a pathogenic mutation (germline and/or somatic) in a homologous recombination gene (HRRm) which include BRCA1, BRCA2, ATM, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D or RAD54L. Note: Continue androgen deprivation therapy (ADT) to maintain castrate levels of serum testosterone (<50 ng/dL). (2) Consider inclusion of olaparib in men who have an HRRm and have progressed on prior treatment with enzalutamide and/or abiraterone regardless of prior docetaxel therapy. (3) Patients with PPP2R2A mutations in the PROfound trial experienced an unfavorable risk-benefit profile. Therefore, olaparib is not recommended in patients with a PPP2R2A mutations. (4) The preferred method of selecting patients for rucaparib treatment is somatic analysis of BRCA1 and BRCA2 using a circulating tumor DNA sample.
KRAS p.G13D c.38G>A C NM_033360.2 VAF % 14 DEPTH 2,526	**Not likely to benefit from** — Erlotinib, Osimertinib, or Gefitinib *in Non-small cell lung cancer or Non-small cell carcinoma* — Panitumumab or Cetuximab *in Malignant tumor of colon, Primary adenocarcinoma of colon, Adenocarcinoma of rectum, or Malignant tumor of rectum* **Unfavorable Prognosis in** — Non-small cell lung cancer or Non-small cell carcinoma

STUDY	DISEASE	PARTICIPANT	REPORT DATE	REPORT STATUS
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VARIANT | CLINICAL IMPACT

INTERPRETATION

Survival advantages (OS and PFS, ORR) for G13D mutations over codon 12 and G13D over other mutations were reported in two studies and codon 13 over other KRAS mutations. Recent studies showed conclusive evidence that in addition to mutations in KRAS exon 2, other RAS mutations in KRAS exons 3 and 4 and NRAS exons 2, 3, and 4 were also associated with nonresponse of metastatic CRC to anti-EGFR monoclonal antibody therapy. Patients with CRCs that are KRAS exon 2 nonmutated/wild type but harbor RAS mutations in KRAS exons 3 and 4 or NRAS exons 2, 3, and 4 also have significantly inferior anti-EGFR treatment outcomes benefit compared with those without any RAS mutations. In summary, current evidence indicates that both cetuximab and panitumumab should only be prescribed for patients with metastatic CRCs that are nonmutated/wild type for all known RASactivating mutations.

KRAS

p.G12D
c.35G>A

C

NM_033360.2
VAF % 5.3
DEPTH 2,533

Not likely to benefit from

— Erlotinib, Osimertinib, or Gefitinib *in Non-small cell lung cancer or Non-small cell carcinoma*

— Panitumumab or Cetuximab *in Malignant tumor of colon, Primary adenocarcinoma of colon, Adenocarcinoma of rectum, or Malignant tumor of rectum*

Unfavorable Prognosis in

— Malignant tumor of unknown origin or ill-defined site, Non-small cell lung cancer, Malignant tumor of unknown origin, or Non-small cell carcinoma

INTERPRETATION

Recent studies showed conclusive evidence that in addition to mutations in KRAS exon 2, other RAS mutations in KRAS exons 3 and 4 and NRAS exons 2, 3, and 4 were also associated with nonresponse of metastatic CRC to anti-EGFR monoclonal antibody therapy. Patients with CRCs that are KRAS exon 2 nonmutated/wild type but harbor RAS mutations in KRAS exons 3 and 4 or NRAS exons 2, 3, and 4 also have significantly inferior anti-EGFR treatment outcomes benefit compared with those without any RAS mutations. In summary, current evidence indicates that both cetuximab and panitumumab should only be prescribed for patients with metastatic CRCs that are nonmutated/wild type for all known RASactivating mutations.

STUDY	DISEASE	PARTICIPANT	REPORT DATE	REPORT STATUS
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VARIANT

BRCA2

p.K1691Nfs*15
c.5073delA

C

NM_000059.3
VAF % 31.7
DEPTH 2,118

CLINICAL IMPACT

May benefit from

— Talazoparib or Olaparib *in Malignant tumor of breast, Infiltrating duct carcinoma of breast, Invasive micropapillary carcinoma of breast, Mixed ductal and lobular carcinoma of breast, Mucinous carcinoma of breast, Inflammatory carcinoma of breast, Infiltrating carcinoma with ductal and lobular features, or Infiltrating lobular carcinoma of breast*

— Rucaparib *in Malignant tumor of prostate, Endometrioid carcinoma ovary, Malignant tumor of fallopian tube, Primary malignant clear cell tumor of ovary, Adenocarcinoma of prostate, Malignant tumor of ovary, Carcinosarcoma of ovary, Malignant epithelial tumor of ovary, or Primary malignant neoplasm of the peritoneum*

— Bevacizumab + Olaparib, Bevacizumab-awwb + Olaparib, Niraparib, or Bevacizumab-bvzr + Olaparib *in Endometrioid carcinoma ovary, Malignant tumor of fallopian tube, Primary malignant clear cell tumor of ovary, Malignant tumor of ovary, Carcinosarcoma of ovary, Malignant epithelial tumor of ovary, or Primary malignant neoplasm of the peritoneum*

— Olaparib *in Human epidermal growth factor 2 negative carcinoma of breast, Malignant tumor of prostate, Adenocarcinoma of pancreas, Endometrioid carcinoma ovary, Malignant tumor of fallopian tube, Primary malignant clear cell tumor of ovary, Adenocarcinoma of prostate, Malignant tumor of ovary, Carcinosarcoma of ovary, Malignant epithelial tumor of ovary, or Primary malignant neoplasm of the peritoneum*

— Talazoparib *in Human epidermal growth factor 2 negative carcinoma of breast*

Unfavorable Prognosis in

— Malignant tumor of prostate or Primary malignant neoplasm of prostate

INTERPRETATION

(1) Olaparib is recommended as a single agent therapy (preferred regimen) for recurrent or stage IV (M1) human epidermal growth factor receptor 2 (HER2)-negative, BRCA 1/2-germline mutated disease that is (a) hormone receptor-negative, (b) hormone receptor-positive with visceral crisis or endocrine therapy refractory. (2) Olaparib is recommended as a single agent therapy (preferred regimen) for recurrent or stage IV (M1) human epidermal growth factor receptor 2 (HER2)-positive, BRCA1/2-germline mutated disease that is (a) hormone receptor-negative, (b) hormone receptor-positive with or without endocrine therapy. (3) While olaparib and talazoparib are FDA indicated in HER2-negative disease, the NCCN Panel supports use in any breast cancer subtype associated with germline BRCA1/2 mutations. (4)

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VARIANT	CLINICAL IMPACT

INTERPRETATION

Indications for and recommendations regarding chemotherapy, HER2-targeted therapy, immunotherapy, and PARP inhibitors for advanced breast cancer in men are similar to those for advanced breast cancer in women.

BRCA2

p.N1784Tfs*7
c.5351delA

C

NM_000059.3
VAF % 35.5
DEPTH 1,989

May benefit from

— Talazoparib or Olaparib *in Malignant tumor of breast, Infiltrating duct carcinoma of breast, Invasive micropapillary carcinoma of breast, Mixed ductal and lobular carcinoma of breast, Mucinous carcinoma of breast, Inflammatory carcinoma of breast, Infiltrating carcinoma with ductal and lobular features, or Infiltrating lobular carcinoma of breast*

— Rucaparib *in Malignant tumor of prostate, Endometrioid carcinoma ovary, Malignant tumor of fallopian tube, Primary malignant clear cell tumor of ovary, Adenocarcinoma of prostate, Malignant tumor of ovary, Carcinosarcoma of ovary, Malignant epithelial tumor of ovary, or Primary malignant neoplasm of the peritoneum*

— Bevacizumab + Olaparib, Bevacizumab-awwb + Olaparib, Niraparib, or Bevacizumab-bvzr + Olaparib *in Endometrioid carcinoma ovary, Malignant tumor of fallopian tube, Primary malignant clear cell tumor of ovary, Malignant tumor of ovary, Carcinosarcoma of ovary, Malignant epithelial tumor of ovary, or Primary malignant neoplasm of the peritoneum*

— Olaparib *in Human epidermal growth factor 2 negative carcinoma of breast, Malignant tumor of prostate, Adenocarcinoma of pancreas, Endometrioid carcinoma ovary, Malignant tumor of fallopian tube, Primary malignant clear cell tumor of ovary, Adenocarcinoma of prostate, Malignant tumor of ovary, Carcinosarcoma of ovary, Malignant epithelial tumor of ovary, or Primary malignant neoplasm of the peritoneum*

— Talazoparib *in Human epidermal growth factor 2 negative carcinoma of breast*

Unfavorable Prognosis in

— Malignant tumor of prostate or Primary malignant neoplasm of prostate

INTERPRETATION

(1) Olaparib is recommended as a single agent therapy (preferred regimen) for recurrent or stage IV (M1) human epidermal growth factor receptor 2 (HER2)-negative, BRCA 1/2-germline mutated disease that is (a) hormone receptor-negative, (b) hormone receptor-positive with visceral crisis or endocrine therapy refractory. (2)

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VARIANT	CLINICAL IMPACT

INTERPRETATION

Olaparib is recommended as a single agent therapy (preferred regimen) for recurrent or stage IV (M1) human epidermal growth factor receptor 2 (HER2)-positive, BRCA1/2-germline mutated disease that is (a) hormone receptor-negative, (b) hormone receptor-positive with or without endocrine therapy. (3) While olaparib and talazoparib are FDA indicated in HER2-negative disease, the NCCN Panel supports use in any breast cancer subtype associated with germline BRCA1/2 mutations. (4) Indications for and recommendations regarding chemotherapy, HER2-targeted therapy, immunotherapy, and PARP inhibitors for advanced breast cancer in men are similar to those for advanced breast cancer in women.

BRCA2

p.I2675Dfs*6
c.8021dupA

C

NM_000059.3
VAF % 8.2
DEPTH 2,451

May benefit from

— Talazoparib or Olaparib *in Malignant tumor of breast, Infiltrating duct carcinoma of breast, Invasive micropapillary carcinoma of breast, Mixed ductal and lobular carcinoma of breast, Mucinous carcinoma of breast, Inflammatory carcinoma of breast, Infiltrating carcinoma with ductal and lobular features, or Infiltrating lobular carcinoma of breast*

— Rucaparib *in Malignant tumor of prostate, Endometrioid carcinoma ovary, Malignant tumor of fallopian tube, Primary malignant clear cell tumor of ovary, Adenocarcinoma of prostate, Malignant tumor of ovary, Carcinosarcoma of ovary, Malignant epithelial tumor of ovary, or Primary malignant neoplasm of the peritoneum*

— Bevacizumab + Olaparib, Bevacizumab-awwb + Olaparib, Niraparib, or Bevacizumab-bvzr + Olaparib *in Endometrioid carcinoma ovary, Malignant tumor of fallopian tube, Primary malignant clear cell tumor of ovary, Malignant tumor of ovary, Carcinosarcoma of ovary, Malignant epithelial tumor of ovary, or Primary malignant neoplasm of the peritoneum*

— Olaparib *in Human epidermal growth factor 2 negative carcinoma of breast, Malignant tumor of prostate, Adenocarcinoma of pancreas, Endometrioid carcinoma ovary, Malignant tumor of fallopian tube, Primary malignant clear cell tumor of ovary, Adenocarcinoma of prostate, Malignant tumor of ovary, Carcinosarcoma of ovary, Malignant epithelial tumor of ovary, or Primary malignant neoplasm of the peritoneum*

— Talazoparib *in Human epidermal growth factor 2 negative carcinoma of breast*

Unfavorable Prognosis in

— Malignant tumor of prostate or Primary malignant neoplasm of prostate

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VARIANT | CLINICAL IMPACT

INTERPRETATION

(1) Olaparib is recommended as a single agent therapy (preferred regimen) for recurrent or stage IV (M1) human epidermal growth factor receptor 2 (HER2)-negative, BRCA 1/2-germline mutated disease that is (a) hormone receptor-negative, (b) hormone receptor-positive with visceral crisis or endocrine therapy refractory. (2) Olaparib is recommended as a single agent therapy (preferred regimen) for recurrent or stage IV (M1) human epidermal growth factor receptor 2 (HER2)-positive, BRCA1/2-germline mutated disease that is (a) hormone receptor-negative, (b) hormone receptor-positive with or without endocrine therapy. (3) While olaparib and talazoparib are FDA indicated in HER2-negative disease, the NCCN Panel supports use in any breast cancer subtype associated with germline BRCA1/2 mutations. (4) Indications for and recommendations regarding chemotherapy, HER2-targeted therapy, immunotherapy, and PARP inhibitors for advanced breast cancer in men are similar to those for advanced breast cancer in women.

BRCA1

p.R1443*
c.4327C>T

C

NM_007300.3
VAF % 26.6
DEPTH 3,302

May benefit from

— Talazoparib or Olaparib *in Human epidermal growth factor 2 negative carcinoma of breast or Malignant tumor of breast*

— Talazoparib *in Infiltrating duct carcinoma of breast, Invasive micropapillary carcinoma of breast, Mixed ductal and lobular carcinoma of breast, Mucinous carcinoma of breast, Inflammatory carcinoma of breast, Infiltrating carcinoma with ductal and lobular features, or Infiltrating lobular carcinoma of breast*

— Rucaparib *in Malignant tumor of prostate, Malignant tumor of fallopian tube, Endometrioid carcinoma ovary, Primary malignant clear cell tumor of ovary, Adenocarcinoma of prostate, Malignant tumor of ovary, Malignant epithelial tumor of ovary, Primary malignant neoplasm of the peritoneum, or Carcinosarcoma of ovary*

— Olaparib *in Invasive micropapillary carcinoma of breast, Mixed ductal and lobular carcinoma of breast, Primary malignant clear cell tumor of ovary, Adenocarcinoma of prostate, Malignant epithelial tumor of ovary, Carcinosarcoma of ovary, Malignant tumor of prostate, Adenocarcinoma of pancreas, Infiltrating duct carcinoma of breast, Malignant tumor of fallopian tube, Endometrioid carcinoma ovary, Mucinous carcinoma of breast, Inflammatory carcinoma of breast, Infiltrating carcinoma with ductal and lobular features, Infiltrating lobular carcinoma of breast, Malignant tumor of ovary, or Primary malignant neoplasm of the peritoneum*

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VARIANT | CLINICAL IMPACT

— Bevacizumab + Olaparib, Bevacizumab-awwb + Olaparib, Niraparib, or Bevacizumab-bvzr + Olaparib *in Endometrioid carcinoma ovary, Malignant tumor of fallopian tube, Primary malignant clear cell tumor of ovary, Malignant tumor of ovary, Carcinosarcoma of ovary, Malignant epithelial tumor of ovary, or Primary malignant neoplasm of the peritoneum*

Unfavorable Prognosis in

— Malignant tumor of prostate

INTERPRETATION

(1) Talazoparib is recommended as a single agent therapy (preferred regimen) for recurrent or stage IV (M1) human epidermal growth factor receptor 2 (HER2) -negative, BRCA 1/2-germline mutated disease that is (a) hormone receptor-negative, (b) hormone receptor-positive with visceral crisis or endocrine therapy refractory. (2) Talazoparib is recommended as a single agent therapy (preferred regimen) for recurrent or stage IV (M1) human epidermal growth factor receptor 2 (HER2)-positive, BRCA1/2-germline mutated disease that is (a) hormone receptor-negative, (b) hormone receptor-positive with or without endocrine therapy. (3) While olaparib and talazoparib are FDA indicated in HER2-negative disease, the NCCN Panel supports use in any breast cancer subtype associated with germline BRCA1/2 mutations. (4) Indications for and recommendations regarding chemotherapy, HER2-targeted therapy, immunotherapy, and PARP inhibitors for advanced breast cancer in men are similar to those for advanced breast cancer in women.

SMARCA4

p.S122Lfs*7
c.363dupC

C

NM_001128849.1
VAF % 11
DEPTH 2,625

Diagnostic of

— Undifferentiated sarcoma

INTERPRETATION

(1) PRINCIPLES OF PATHOLOGY AND MOLECULAR ANALYSIS: SMARCA4 mutation in a small subset of Undifferentiated Uterine Sarcoma. (2) HALLMARKS FOR HISTOLOGIC DIAGNOSIS: Infiltrative sheets of pleomorphic epithelioid and/or spindle cells. SMARCA4-deficient subset consists of epithelioid/rhabdoid cells associated with myxoid matrix. lymphovascular space invasion (LVSI), high MI (mitotic index), and necrosis are common (3) TESTS NEEDED TO CONFIRM DIAGNOSIS: IHC panel of CD10, BCOR, cyclin D1, desmin, SMA, pan-CK, EMA, BRG1, INI1, pan-Trk, ALK, HMB45, melan

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VARIANT	CLINICAL IMPACT

INTERPRETATION

A, SOX10, and STAT6 is recommended to exclude other tumor types. Absence of ESS associated fusions by FISH and/or targeted RNA sequencing is recommended. Absent CK expression and BRG1 loss (SMARCA4) and/or SMARCA4 mutation detectable by DNA sequencing is confirmatory of SMARCA4-deficient tumors. (4) ER and/or PR expression may correlate with improved survival. MI >=11/mm2 is associated with decreased survival.

RUNX1

p.M267I
c.801G>A

C

NM_001754.4
VAF % 9.2
DEPTH 4,046

Unfavorable Prognosis in

— Acute myeloid leukemia or Acute myeloid leukemia, disease

INTERPRETATION

(1) Non- acute promyelocytic leukemia AML (Non-APL AML) with the genetic abnormality mutated RUNX1 is categorized as Poor/Adverse risk category. These markers should not be used as an adverse prognostic marker if they co-occur with favorable-risk AML subtypes. (2) AML (Acute Myeloid Leukemia) with RUNX1 mutation is associated with a poorer prognosis. (3) Other candidate genes that are associated with an adverse impact on outcome are TET2 and RUNX1. (4) The runt-related transcription factor 1 (RUNX1) gene, encoding a myeloid transcription factor, is mutated in approximately 10% of de novo AML cases and associated with adverse prognoses. (5) In a study examining the impact of multiple RUNX1 mutations and loss of wild-type RUNX1 in AML, both loss of wild-type RUNX1 (OS, 5 months) and having more than or equal to 1 RUNX1 mutation (14 months) had an adverse impact on prognosis compared to 1 RUNX1 mutation (22 months; P < .002 and .048, respectively). (6) Both NCCN and the ELN classify patients with wild-type NPM1 and FLT3-ITD high, mutated TP53, mutated RUNX1, or mutated ASXL1 as having poor risk. However, mutated RUNX1 or ASXL1 should not be used as poor-risk prognostic markers if they co-occur with favorable-risk AML subtypes. (7) Prognostic impact of the biomarker is treatment-dependent and may change with new therapies.

BCOR

p.Q1208Tfs*8
c.3621dupA

Unfavorable Prognosis in

— Myelodysplastic syndrome (clinical) or Myelodysplastic syndrome

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VARIANT	CLINICAL IMPACT
C NM_001123385.1 VAF % 13.7 DEPTH 1,662	INTERPRETATION Genes frequently somatically mutated in MDS include BCOR. Nonsense or Frameshift or Splice Site mutations in BCOR are associated with a poor prognosis.

Other Biomarkers

BIOMARKER	CLINICAL IMPACT
TMB	
485.2 muts/Mb	INTERPRETATION
MSI	
7.3% Unstable Sites	INTERPRETATION

POTENTIAL CLINICAL TRIALS

No relevant clinical trials were reported.

CLASSIFICATION AND LEVELS OF EVIDENCE

The variant classification system used in this report is based on joint consensus recommendations of the Association for Molecular Pathology, American Society of Clinical Oncology, and the College of American Pathologists (J Mol Diagn 2017, 19:4-23). Tiers IA, IB, IIC, IID, III and IV describe variant categories of descending clinical significance in the patient. Variants in Tier IV are not reported in accordance with the consensus recommendations.

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IA	IB	IIC	IID
Variant of strong clinical significance, Level A evidence (FDA approved therapy or practice guideline in patient's tumor type)	Variant of strong clinical significance, Level B Evidence (consensus in the field based on well-powered studies in patient's tumor type)	Variant of potential clinical significance, Level C evidence (FDA approved therapy or practice guideline in other tumor type(s), evidence from multiple small published studies, or based on availability of investigational therapies)	Variant of potential clinical significance, Level D evidence (case reports or preclinical studies)

III	Variant of uncertain clinical significance
IV	Benign or likely benign variant

TEST DETAILS

REPORTED GENES

A total of 523 genes were subjected to targeted next generation sequencing analysis. Details available upon request.

CGW VERSION

CGW_v6.13.1

DATABASE DETAILS

The versions, releases, builds, dates of the following databases were used to generate this report.

— Genomic Build: GRCh37.p13
— Genomic Annotation Sources: NCBI RefSeq v105
— dbSNP: 149
— NHLBI ESP: v.0.0.30
— ClinVar: 20190603
— COSMIC: v89
— ExAC: v1.0
— gnomAD: r2.1
— dbNSFP: 3.5c

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METHODOLOGY

Assay Methods: The test was performed using the Illumina TruSight ™ Oncology 500 (TSO500) targeted hybrid-capture based next generation sequencing assay. It employs Unique molecular identifiers (UMI) to enable detection of variants, present in solid tumor, formalin-fixed paraffin-embedded (FFPE) tumor, or ctDNA samples, at low VAFs with a high degree of sensitivity and specificity. TSO500 is designed to detect multiple classes of mutations including single-nucleotide variants (SNVs), multi-nucleotide variants (<3bp), small Insertions (1-18bp)/Deletions (1-27bp) and Copy Number Variants (CNVs). The assay also detects, quantitatively, microsatellite instability (MSI) and tumor mutational burden (TMB). Fusions are detected in RNA (TSO500 Solid Tumor reports only) and Gene Rearrangements are detected from ctDNA (TSO500 ctDNA reports only). DNA and RNA (TSO500 Solid Tumor reports) or ctDNA (ctDNA reports) extractions are performed and RNA is then reverse transcribed to cDNA. The genomic DNA and cDNA (TSO500 Solid Tumor reports) or ctDNA (TSO500 ctDNA reports) are sheared to prepare sequencing libraries. The regions of interest are hybridized to biotinylated probes, magnetically pulled down with streptavidin-coated beads, and eluted to enrich the library pool. Finally, libraries are normalized, then pooled and sequenced on an Illumina NextSeq or NovaSeq instrument.

Secondary Analysis Methods: The ctDNA from liquid biopsy data are analyzed using Illumina TSO500 ctDNA pipeline v1.1.0 at Discovery Life Sciences. Further processing for interpretation and annotation is done using a customized analysis workflow within the Clinical Genomics Workspace software platform from PierianDx.

Variant Calling: Variants are reported according to HGVS nomenclature (www.hgvs.org/mutnomen) and classified as per the AMP classification system into tiers IA, IB, IIC, IID, III and IV. These tiers are stratified by clinical utility ('actionability' for clinical decision-making as to diagnosis, prognosis, treatment options, and carrier status) and previously reported data in the medical literature. Variations found in gnomAD (https://gnomad.broadinstitute.org/) that have ≥1% minor allele frequency (except those that are also in ClinVar denoted as clinically relevant, used in a clinical diagnostic assay, or reported as a mutation in a publication) are classified as known polymorphisms. Small variant calls in the HLA-A, KMT2B, KMT2C, and KMT2D genes are filtered out due to potential mis-mapping as a result of sequence homology with other genomic regions.

Notes:

- This assay does not detect complex structural alterations or large indels, with the exception of a subset of clinically relevant complex EGFR exon 19 indels that are specifically targeted. Variants located outside of targeted capture regions will not be detected.

- It is possible that pathogenic variants may not be reported by one or more of the tools because of the parameters used. However, tool parameters were optimized to maximize specificity and sensitivity.

- Variant Allele Fraction (VAF) cutoffs for the results provided in this report were set at 0.1% based on RUO verification studies performed at Discovery Life Sciences.

DISCLAIMER

The RUO assay was performed using ctDNA from liquid biopsy; it is therefore not possible to unequivocally determine whether variants detected are somatic or germline in origin unless a matched germline normal sample was analyzed using the same RUO assay and a tumor/normal pair VCF was generated prior to PierianDx interpretation or, in cases

where tumor-only sample was analyzed, a tumor-only informed bioinformatics pipeline was used to generate the VCF prior to PierianDx interpretation.

PATIENT AND ORDER DETAILS

PATIENT

SEX

ETHNICITY

RACE

SPECIMEN

SPECIMEN TYPE

Deoxyribonucleic acid sample

EXT. SPECIMEN ID

% TUMOR IN SELECTED AREA

CASE

REVIEW STATUS

Final

DATE ACCESSIONED

02/23/2021 17:21

DATE REPORTED

ACCESSION NUMBER

6158-SL-0056