# Oncology Pharmacogenetic Genotyping Panel

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About the test

Pharmacogenetics is the study of genetic determinants of interindividual drug response variability, and accumulating evidence supporting clinical utility for certain pharmacogenetic variants has prompted recent genotype-directed clinical practice recommendations for selected gene/drug pairs. Sema4 has designed and validated an Oncology Pharmacogenetic (PGx) Genotyping panel that is intended to help physicians prescribe selected medications that can be influenced by interindividual genetic variability. The panel includes 64 variants in 7 genes. These genes and variants in the clinical PGx panel inform on 16 medications used to treat certain cancers.

The Sema4 Oncology PGx Genotyping Panel is intended for adults and older children. Please note that a Sema4 Pediatric Pharmacogenetic (PGx) Genotyping panel is also available for children.

Indications

The Sema4 Oncology PGx Genotyping panel is intended to provide medication recommendations and drug response information to health care providers based on pharmacogenetic results. The information provided from this panel may help physicians make more informed management decisions regarding drug administration. Please note that any modification of therapy should only be performed as directed by a healthcare professional.

Testing methods, sensitivity, and limitations

Targeted genotyping is performed for this test using multiplex Polymerase Chain Reaction (PCR) and multiplex Single Base Extension (SBE) reaction with Agena® SpectroCHIP® II on a MassARRAY® Analyzer 4 system. In addition, Multiplex ligation-dependent probe amplification (MLPA) serves as an adjunct test to interrogate copy number variants in the CYP2D6 gene. This panel also includes targeted interrogation of the UGT1A1 promoter dinucleotide repeat variant (*28), which is genotyped by PCR and capillary electrophoresis. Resources including the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines, FDA drug label recommendations, and peer-reviewed literature are used in the interpretation of these PGx testing results.

The analytical sensitivity and specificity of this assay is estimated to be greater than 99%. Only genes and variants included in this panel will be detected and reported. This test does not detect all the known alleles that result in altered or inactive gene function. This test does not account for all genetic variants in tested individuals. Absence of a detected gene variant with this panel does not rule out the possibility that a patient will have an atypical drug response phenotype, which could be due to an untested genetic variant or other factors such as drug-drug interactions, comorbidities, and/or other clinical and environmental variables.

Turnaround time

Results are reported to the referring physician within 7-10 business days from the receipt of the specimen.

Specimen and shipping requirements

**Blood samples:** Two lavender-top (EDTA) or two yellow-top (ACD-A or ACD-B) tubes, 5-10 mL of blood from the patient are required.

**Extracted DNA samples:** We request 50 µL DNA (50-250 ng/µL) or at minimum require 20 µL DNA (50-250 ng/µL). Causes for rejection include impurities in the test or reference DNA samples, including NaCl or KCl (>40 mM) and other salts, phenol, ethanol, heparin, EDTA (>1.5 mM), and Fe, contaminated DNA, and low concentration of DNA (<20 ng/µL).
Saliva samples: We can accept saliva specimens upon request. Saliva samples should be collected in Oragene DNA (OG-500) kits by DNA Genotek. Please contact our laboratory to obtain saliva kits.

Tubes of blood should be kept and shipped refrigerated or at room temperature (PLEASE DO NOT FREEZE).

Customer services and genetic counseling

Include the following with each sample:
- Completed and signed test requisition form and informed consent
- Billing information or payment (include copy of insurance card)
- Contact information for referring physician
- Testing to be performed (Comprehensive PGx Panel)
- Indication for testing, patient’s family history, ethnic background and prior relevant test results

Send same day or overnight (check for morning delivery) to:
Sema4
1428 Madison Avenue, Atran 2-25
New York, NY 10029

Contact:
labgeneticcounselors@sema4genomics.com
Tel: 212-241-7518
Fax: 212-241-0139

Disclaimer
This test was developed and its performance characteristics were determined by Sema4 and was considered acceptable for patient testing. It has not been cleared or approved by the FDA. The FDA has determined that such clearance or approval is not necessary. This type of mutation analysis generally provides highly accurate genotype information for single nucleotide variants and small insertion/deletion variants. Despite this level of accuracy, it should be kept in mind that there are many potential sources of diagnostic error, including misidentification of samples, rare polymorphisms, or other rare genetic variants that interfere with analysis. In addition, families should understand the limitations of the testing and that rare diagnostic errors may occur for the reasons described.

References

Medication list

**Azathioprine**

Azathioprine (Imuran®) is classified as an immunosuppressant agent indicated for the treatment of renal transplantation, rheumatoid arthritis and several “off-label” uses (Crohn’s disease, Multiple Sclerosis, Psoriasis, Ulcerative Colitis). Azathioprine is an imidazolyl derivative of mercaptopurine; metabolites are incorporated into replicating DNA and halt replication; also block the pathway for purine synthesis. The 6-thioguanine nucleotide metabolites appear to mediate the majority of azathioprine’s immunosuppressive and toxic effects. TPMT is an enzyme that is involved in the metabolism of azathioprine to its inactive metabolites. Patients with intermediate thiopurine S-methyl transferase (TPMT) activity may be at an increased risk of myelotoxicity if receiving conventional doses of IMURAN. Patients with low or absent TPMT activity are at an increased risk of developing severe, life-threatening myelotoxicity if receiving conventional doses of IMURAN. TPMT genotyping or phenotyping can help identify patients who are at an increased risk for developing IMURAN toxicity. Reduced dosing (30-70%) in those individuals who have intermediate activity is recommended. Those who have low or deficient TPMT activity are recommended to use alternative agents, or drastically reduce dose (10-fold reduction with thrice weekly dosing instead of daily).

**References:** PMID: 18253145, 21270794, 23422873

**Belinostat**

Belinostat (Beleodaq) is an antineoplastic agent and a histone deacetylase inhibitor. Belinostat is indicated for the treatment of Peripheral T-cell lymphoma. Belinostat catalyzes acetyl group removal from protein lysine residues (of histone and some nonhistone proteins). Inhibition of histone deacetylase results in accumulation of acetyl groups, leading to cell cycle arrest and apoptosis. Belinostat has preferential cytotoxicity toward tumor cells versus normal cells. Belinostat is primarily metabolized by UGT1A1. The FDA-approved drug label for belinostat states: Because belinostat is primarily (80-90%) metabolized by UGT1A1, the clearance of belinostat could be decreased in patients with reduced UGT1A1 activity (e.g., patients with UGT1A1*28 allele). Reduce the starting dose of Beleodaq to 750 mg/m2 in patients known to be homozygous for the UGT1A1*28 allele to minimize dose limiting toxicities.

**References:** FDA-approved drug label

**Capecitabine**

Capecitabine (Xeloda) is an antineoplastic agent known as a Pyrimidine analog. It is used in the treatment of metastatic breast cancer, colorectal cancer and as adjuvant therapy in Dukes’ C colon cancer. Capecitabine is a prodrug of fluorouracil. It undergoes hydrolysis in the liver and tissues to form fluorouracil which is the active moiety. Fluorouracil is a fluorinated pyrimidine antimetabolite that inhibits thymidylate synthetase, blocking the
methylation of deoxyuridylic acid to thymidylic acid, interfering with DNA, and to a lesser degree, RNA synthesis. Fluorouracil appears to be phase specific for the G1 and S phases of the cell cycle. Detoxifying metabolism of fluoropyrimidines requires dihydropyrimidine dehydrogenase (DPD, encoded by the DPYD gene), and reduced or absent activity of this enzyme can result in severe, and sometimes fatal toxicity. Current guidelines state that those individuals who are found to be heterozygous variant or having intermediate DPD activity it is recommended to start with at least a 50% reduction in starting dose. Individuals who are homozygous variant with complete DPD deficiency it is recommended to use an alternate drug.

References: PMID: 23988873, 18253145

Dabrafenib

Dabrafenib (Tafinlar) is a tyrosine kinase inhibitor. It is indicated for the Melanoma, metastatic or unresectable (with BRAF V600E or V600K mutation), and Non-small cell lung cancer, metastatic, relapsed or refractory (with BRAF V600E Mutation) (off label). Dabrafenib selectively inhibits some mutated forms of the protein kinase B-raf (BRAF). BRAF V600 mutations result in constitutive activation of the BRAF pathway; through BRAF inhibition, dabrafenib inhibits tumor cell growth. The FDA approved label for dabrafenib states; patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency may be at risk for hemolytic anemia when administered dabrafenib; use with caution and closely observe for signs/symptoms of hemolytic anemia.

References: FDA reference ID 3315786

Erlotinib

Erlotinib (Tarceva) is a drug used to treat non-small cell lung cancer, pancreatic cancer and several other types of cancer. The mechanism of clinical antitumor action of erlotinib is not fully characterized. Erlotinib inhibits the intracellular phosphorylation of tyrosine kinase associated with the epidermal growth factor receptor (EGFR). Specificity of inhibition with regard to other tyrosine kinase receptors has not been fully characterized. EGFR is expressed on the cell surface of normal cells and cancer cells. Erlotinib is metabolized primarily by CYP3A4 and to a lesser extent by CYP1A2, and the extrahepatic isoform CYP1A1, in vitro. The inhibition of glucuronidation may cause interactions with medicinal products which are substrates of UGT1A1 and exclusively cleared by this pathway. Patients with low expression levels of UGT1A1 or genetic glucuronidation disorders (e.g. Gilbert’s disease) may exhibit increased serum concentrations of bilirubin and must be treated with caution.

References: FDA drug labeling on Dailymed, EMA Drug labeling, DrugBank

Fluorouracil

Fluorouracil (Adrucil, Efudex) is a widely used antineoplastic agent (pyrimidine analog) indicated for the treatment of a number of different cancers, including breast, colorectal, gastric and pancreatic. As a topical agent fluorouracil is indicated for actinic or solar keratosis, and also for superficial basal cell carcinoma. Fluorouracil inhibits thymidylate synthetase, blocking the methylation of deoxyuridylic acid to thymidylic acid, interfering with DNA, and to a lesser degree, RNA synthesis. Fluorouracil appears to be phase specific for the G1 and S phases of the cell cycle. Detoxifying metabolism of fluoropyrimidines requires dihydropyrimidine dehydrogenase (DPD, encoded by the DPYD gene), and reduced or absent activity of this enzyme can result in severe, and sometimes fatal toxicity. Current guidelines state that those individuals who are found to be heterozygous variant or having intermediate DPD activity it is recommended to start with at least a 50% reduction in starting dose. Individuals who are homozygous variant with complete DPD deficiency it is recommended to use an alternate drug.

References: PMID: 21412232, 23988873

Gefitinib

Gefitinib (IRESSA) is a tyrosine kinase inhibitor indicated for the first-line treatment of patients with metastatic non-small cell lung cancer (NSCLC) whose tumors have epidermal growth factor receptor (EGFR) exon 19 deletions or exon 21 (L858R) substitution mutations as detected by an FDA-approved test. Gefitinib inhibits the epidermal growth
factor receptor (EGFR) tyrosine kinase by binding to the adenosine triphosphate (ATP)-binding site of the enzyme. Thus the function of the EGFR tyrosine kinase in activating the Ras signal transduction cascade is inhibited; and malignant cells are inhibited. CYP2D6 metabolizes gefitinib to O-desmethyl gefitinib in vitro. In healthy CYP2D6 poor metabolizers, O-desmethyl gefitinib concentration was not measurable and the mean exposure to gefitinib was 2-fold higher as compared to the extensive metabolizers. This increase in exposure in CYP2D6 poor metabolizers may be clinically important because some adverse drug reactions are related to higher exposure of gefitinib. No dose adjustment is recommended in patients with a known CYP2D6 poor metabolizer genotype, but these patients should be closely monitored for adverse reactions.

**References:** FDA reference ID 3791123

**Irinotecan**

Irinotecan (Camptosar) is an anticancer agent belonging to a class of drugs known as topoisomerase I inhibitors. Irinotecan is indicated for the treatment of metastatic colorectal cancer as a single-agent and also in combination with fluorouracil and leucovorin. Irinotecan inhibits the action of topoisomerase I. Irinotecan prevents re-ligation of the DNA strand by binding to topoisomerase I-DNA complex. The formation of this ternary complex interferes with the moving replication fork, which induces replication arrest and lethal double-stranded breaks in DNA. As a result, DNA damage is not efficiently repaired and apoptosis (programmed cell death) occurs. Irinotecan as part of its metabolism undergoes conjugation by UDP-glucuronosyl transferase 1A1 (UGT1A1) to form a glucuronide metabolite. According to the FDA approved drug label, Patients homozygous for the UGT1A1*28 allele, a genetic polymorphism present in approximately 10% of the North American population that leads to reduced UGT1A1 enzyme activity, are at increased risk for neutropenia resulting from treatment with irinotecan. Individuals heterozygous for the UGT1A1*28 allele may be at increased risk for neutropenia. Guidelines suggest reducing intitial doses by 25-30% in those individuals who are homozygous carriers of the *28 allele.

**References:** FDA reference ID 3158344, PMID: 25817555, 21412232

**Mercaptopurine**

Mercaptopurine (Purinethol) is an antimetabolite (purine analog) and immunosuppressant agent. Mercaptopurine is indicated for the treatment acute lymphoblastic leukemia (ALL), and “off-label” for Crohn’s disease, and Ulcerative Colitis. Mercaptopurine is a purine antagonist which inhibits DNA and RNA synthesis; acts as false metabolite and is incorporated into DNA and RNA, eventually inhibiting their synthesis; specific for the S phase of the cell cycle. Thiopurine S-methyltransferase (TPMT) inactivates mercaptopurine, leaving less parent drug available to form thioguanine nucleotides TGNs, the major active metabolite. Guidelines suggest that those individuals who are heterozygote (intermediate activity), having one functional allele and one nonfunctional allele, starting doses be reduced by 30-70% of full dose. In those individuals who are homozygous variant, having low or deficient TPMT activity, starting doses of mercaptopurine should be drastically reduced by 10-fold and given only 3 times a week as opposed to daily. Doses should be adjusted according to myelosuppression and disease specific guidelines.

**References:** PMID: 28520348, 23422873, 21412232

**Nilotinib**

Nilotinib (Tasigna) is an antineoplastic agent prescribed for treatment of various leukemias, including chronic myeloid leukemia. Nilotinib is a selective tyrosine kinase inhibitor that targets BCR-ABL kinase, c-KIT and platelet derived growth factor receptor (PDGFR); does not have activity against the SRC family. It inhibits BCR-ABL mediated proliferation of leukemic cell lines by binding to the ATP-binding site of BCR-ABL and inhibiting tyrosine kinase activity. Nilotinib is indicated for use in patients diagnosed with Philadelphia chromosome positive (presence of a BCR-ABL1 gene fusion) chronic myeloid leukemia, due to its mechanism of action. Individuals with variants in UGT1A1 gene are at an increased risk of hyperbilirubinemia when taking nilotinib. Current guidelines recommend testing for Philadelphia chromosome.

**References:** FDA Reference ID: 3380785
Paclitaxel

Paclitaxel (Abraxane®) is used in the treatment of Kaposi’s sarcoma and cancer of the lung, ovarian, and breast. Abraxane® is specifically indicated for the treatment of metastatic breast cancer and locally advanced or metastatic non-small cell lung cancer. Paclitaxel promotes microtubule assembly by enhancing the action of tubulin dimers, stabilizing existing microtubules, and inhibiting their disassembly, interfering with the late G2 mitotic phase, and inhibiting cell replication. In addition, the drug can distort mitotic spindles, resulting in the breakage of chromosomes. Paclitaxel may also suppress cell proliferation and modulate immune response. Paclitaxel is metabolized through the CYP2C8 and CYP3A4 enzyme system. Reduced function polymorphisms in the CYP2C8 gene could lead to increased plasma concentrations of paclitaxel at standard doses and increase risk of paclitaxel induced peripheral neuropathy and anemia.

References: DrugBank, DailyMed FDA-approved drug label, PMID: 27736846, 23413280, 25495407

Pazopanib

Pazopanib (Votrient) is an antineoplastic agent prescribed for treatment of advanced renal cell cancer and advanced soft tissue sarcoma (in patients previously treated with chemotherapy). Pazopanib is a tyrosine kinase (multikinase) inhibitor; limits tumor growth via inhibition of angiogenesis by inhibiting cell surface vascular endothelial growth factor receptors, platelet-derived growth factor receptors, fibroblast growth factor receptor, cytokine receptor, interleukin-2 receptor inducible T-cell kinase, leukocyte-specific protein tyrosine kinase, and transmembrane glycoprotein receptor tyrosine kinase. Patients with the UGT1A1 *28/*28 genotype had a significantly increased incidence of hyperbilirubinemia when taking pazopanib, as compared to those with the *1/*1 or *1/*28 genotype.

References: FDA Reference ID: 3968512

Rasburicase

Rasburicase (Elitek) is a recombinant urate-oxidase agent prescribed for initial management of plasma uric acid levels in pediatric and adult patients with leukemia, lymphoma, and solid tumor malignancies who are receiving anticancer therapy expected to result in tumor lysis and subsequent elevation of plasma uric acid. Rasburicase catalyzes enzymatic oxidation of poorly soluble uric acid into an inactive and more soluble metabolite. It is contraindicated in G6PD deficient patients with or without chronic non-spherocytic hemolytic anemia (CNSHA) due to the risk of developing hemolysis. Current guidelines recommend to not administering rasburicase to patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency.

References: PMID: 24787449, FDA-approved drug label
https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/103946s5083lbl.pdf

Tamoxifen

Tamoxifen (Novadex®, Soltamox®) is one of the selective estrogen receptor modulators (SERM) with tissue-specific activities for the treatment and prevention of estrogen receptor positive breast cancer. Tamoxifen acts as an anti-estrogen (inhibiting agent) in the mammary tissue, but as an estrogen (stimulating agent) in cholesterol metabolism, bone density, and cell proliferation in the endometrium. Tamoxifen is extensively metabolized through CYP-450 enzyme system. CYP2D6 being one of the major enzymes to metabolize Tamoxifen. CPIC guidelines exist for giving therapeutic recommendations to those individuals with known CYP2D6 genotypes/phenotype. Those having a poor metabolizer phenotype, the recommendations are to consider hormonal therapy such as an aromatase inhibitor for postmenopausal women or aromatase inhibitor along with ovarian function suppression in premenopausal women, given that these approaches are superior to tamoxifen regardless of CYP2D6 genotype. If aromatase inhibitor use is contraindicated, consideration should be given to use a higher, but FDA approved tamoxifen dose (40mg/day). Avoid CYP2D6 strong to weak inhibitors.

References: DrugBank, UpToDate, PMID: 29385237
Thiopurine (Thioguanine)

Thioguanine (Tabloid) is a thiopurine antineoplastic agent prescribed for acute nonlymphocytic leukemias. Thioguanine has multiple metabolic effects. Its tumor inhibitory properties may be due to one or more of its effects on (a) feedback inhibition of de novo purine synthesis; (b) inhibition of purine nucleotide interconversions; or (c) incorporation into the DNA and the RNA. The net consequence of its actions is a sequential blockade of the synthesis and utilization of the purine nucleotides. There are individuals with an inherited deficiency of the enzyme thiopurine methyltransferase (TPMT) who may be unusually sensitive to the myelosuppressive effects of thioguanine and prone to developing rapid bone marrow suppression following the initiation of treatment. Current guidelines recommend to start with reduced doses of thioguanine for patients with one nonfunctional TPMT allele, or drastically reduced doses for patients with malignancy and two nonfunctional alleles; adjust dose based on degree of myelosuppression and disease-specific guidelines. Consider alternative nonthiopurine immunosuppressant therapy for patients with nonmalignant conditions and two nonfunctional alleles.

References: PMID: 23422873, 21412232