Pharmacogenetic and Pharmacodynamic Testing

Policy

*Please see amendment for Pennsylvania Medicaid at the end of this CPB.*

Cytochrome P450 polymorphisms

A. Aetna considers one genotyping for CYP2C19 polymorphisms medically necessary for persons who have been prescribed clopidogrel (Plavix). Repeat CYP2C19 genotyping has no proven value.

B. Aetna considers one genotyping for CYP2D6 polymorphisms medically necessary for persons who have been prescribed doses of tetrabenazine (Xenazine) greater than 50 mg per day. Repeat CYP2D6 genotyping has no proven value.

C. Aetna considers one genotyping for CYP2D6 polymorphisms medically necessary for persons with Gaucher disease type 1 who are being considered for treatment with eliglustat (Cerdelga). Repeat CYP2D6 genotyping has no proven value.

D. Aetna considers CYP2D6 genotyping experimental and investigational for predicting response to beta blockers.

E. Aetna considers CYP2D6 genotyping experimental and investigational for identifying individuals with Alzheimer's disease with different clinical response to donepezil (Aricept) because its clinical value has not been established.

F. Aetna considers genotyping for CYP2C9 to inform genotype-guided dosing of coumarin derivatives experimental and investigational.

G. Aetna considers genotyping for other cytochrome P450 polymorphisms (diagnostic tests to identify specific genetic variations that may be linked to reduced/enhanced effect or severe side effects of drugs metabolized by the
cytochrome P450 system including opioid analgesics, warfarin, tamoxifen, proton pump inhibitors, antipsychotic medications, and selective serotonin reuptake inhibitors) experimental and investigational because the clinical value of this type of genetic testing has not been established.

H. Aetna considers CYP2C19 polymorphisms testing for fluoxetine experimental and investigational.

- Aetna considers genotyping for HLA-B*1502 medically necessary for persons of Asian ancestry before commencing treatment with carbamazepine (Tegretol).

- Aetna considers HLA-B*5701 screening medically necessary for persons infected with HIV-1 before commencing treatment with abacavir (Ziagen).

- Aetna considers an FDA cleared test to detect the following mutations in the CTFR gene medically necessary for persons with cystic fibrosis who are being considered for treatment with ivacaftor (Kalydeco): G551D, G1244E, G1349D, G178R, G551S, R117H, S1251N, S1255P, S549N, and S549R.

- Aetna considers and FDA cleared test to detect the F508del mutation in the CTFR gene medically necessary for persons with cystic fibrosis who are being considered for treatment with lumacaftor/ivacaftor (Orkambi).

- Aetna considers an FDA-approved test for the anaplastic lymphoma kinase (ALK) fusion gene (e.g., the Vysis ALK Break Apart FISH Probe Kit; Ventana ALK (D5F3) CDx Assay) medically necessary for persons who are considering crizotinib (Xalkori), alectinib (Alecensa) or ceritinib (Zykadia) for the treatment of non-small cell lung cancer (NSCLC).

- Aetna considers the MGMT (O(6)-methylguanine-DNA methyltransferase) gene methylation assay medically necessary for predicting response to temozolomide (Temodar) in persons with glioblastoma.

- Aetna considers detecting mutations of the BRAF gene (V600E or V600K) (e.g., the THxID BRAF test, cobas 4800 BRAF V600 mutation test) medically necessary for the following indications:

  - in persons with unresectable or metastatic melanoma who are being considered for treatment with vemurafenib (Zelboraf), dabrafenib (Tafinlar), trametinib (Mekinist), cobimetinib (Cotellic), binimetinib (Mektovi) or encorafenib (Braftovi); or
A. in persons with recurrent or metastatic non-small cell lung cancer who are being considered for treatment with dabrafenib (Tafinlar), pembrolizumab (Keytruda), or vemurafenib (Zelboraf); or
B. in persons with thyroid carcinoma who are being considered for treatment with dabrafenib (Tafinlar) or vemurafenib (Zelboraf).

Aetna considers testing for the presence of virus with the NS3 Q80K polymorphism medically necessary for persons with hepatitis C virus (HCV) genotype 1a infection being considered for treatment with simeprevir (Olysio).

Aetna considers testing for the presence of virus with NS5A resistance-associated polymorphisms medically necessary for persons with hepatitis C virus genotype 1, 3 and 4 infections being considered for treatment with daclatasvir (Daklinza) or elbasvir and grazoprevir (Zepatier).

Aetna considers BRCA testing (e.g., BRACAnalysis CDx) medically necessary for women with advanced epithelial ovarian cancer, fallopian tube or primary peritoneal cancer who have been treated with three or more prior lines of chemotherapy and are being considered for olaparib (Lynparza), and for women with advanced epithelial ovarian cancer, fallopian tube or primary peritoneal cancer who are in complete or partial response to first-line platinum based chemotherapy and are being considered for maintenance treatment with olaparib.

Aetna considers germline BRCA testing (e.g., BRACAnalysis CDx) medically necessary for women with metastatic, human epidermal growth factor receptor 2 (HER2)-negative breast cancer who have previously been treated with chemotherapy in the neoadjuvant, adjuvant or metastatic setting (regardless of family history) and are being considered for olaparib (Lynparza) or talazoparib (Talzenna). Women with hormone receptor (HR)-positive metastatic breast cancer should have been treated with a prior endocrine therapy or be considered inappropriate for endocrine treatment. Somatic (tumor) BRCA testing is considered medically necessary for this indication instead of germline testing or when germline testing is negative.

Aetna considers BRCA testing (e.g., BRACAnalysis CDx) medically necessary for persons with metastatic pancreatic carcinoma whose disease has not progressed on first-line platinum treatment, and are being considered for maintenance treatment with olaparib.

Aetna considers BRCA testing (e.g., BRACAnalysis CDx) medically necessary for men with advanced, recurrent or metastatic prostate cancer who have been treated with
androgen-receptor directed therapy and are being considered for treatment with olaparib (Lynparza).

Aetna considers somatic/tumor BRCA testing (e.g., myChoice CDx) medically necessary for women with advanced epithelial ovarian, fallopian tube or primary peritoneal cancer who have been treated with three or more prior lines of chemotherapy and are being considered for niraparib (Zejula).

Aetna considers somatic/tumor BRCA testing (e.g., myChoice CDx) medically necessary for women with advanced epithelial ovarian, fallopian tube or primary peritoneal cancer who are in a complete or partial response to two or more lines of platinum-based chemotherapy and are being considered for maintenance treatment with niraparib.

Aetna considers somatic/tumor BRCA testing (e.g., FoundationOne CDx) medically necessary for women with epithelial ovarian cancer, fallopian tube or primary peritoneal cancer who are being considered for treatment with rucaparib (Rubraca) after two or more previous lines of chemotherapy, except when the individual has previously tested positive for a deleterious or suspected deleterious germline BRCA mutation. For more information on BRCA testing, see CPB 0227 - BRCA Testing, Prophylactic Mastectomy, and Prophylactic Oophorectomy.

Aetna considers testing for BRAF and NRAS mutations (e.g., cobas KRAS Mutation Test; therascreen KRAS RGQ PCR Kit, Dako EGFR pharmDx Kit) medically necessary for persons with colorectal cancer being considered for treatment with cetuximab (Erbitux) or panitumumab (Vectibix).

Aetna considers the Praxis Extended RAS Panel medically necessary for persons with colorectal cancer who do not have specific mutations in RAS genes [KRAS (exons 2, 3, and 4) and NRAS (exons 2, 3, and 4)] who are being considered for treatment with panitumumab (Vectibix).

Aetna considers genotyping for HLA-B*58:01 medically necessary in Asian persons prior to commencing allopurinol therapy.
Aetna considers testing to detect an epidermal growth factor receptor (EGFR) T790 mutation (e.g., cobas EGFR Mutation Test v2) medically necessary for persons with non-small cell lung cancer being considered for treatment with osimertinib (Tagrisso).

Aetna considers testing to detect an epidermal growth factor receptor (EGFR) exon 19 deletions or exon 21 (L858R) substitution mutations (e.g., cobas EGFR Mutation Test, therascreen EGFR RGQ PCR Kit) medically necessary for persons with non-small cell lung cancer being considered for treatment with osimertinib (Tagrisso), erlotinib (Tarceva), dacomitinib (Vizimpro), gefitinib (Iressa) or afatinib (Gilotrif).

Aetna considers Guardant360CDx testing panel not medically necessary for assessing candidacy of persons with non-small cell lung cancer for treatment with osimertinib (Tagrisso), because there is no proven advantage of the Guardant360 CDx gene panel over targeted EGFR mutation testing or small targeted panels for this indication.

Aetna considers FoundationOne Liquid CDx not medically necessary for assessing candidacy of persons with non-small cell lung cancer for treatment with osimertinib (Tagrisso), erlotinib (Tarceva) or gefitinib (Iressa) because there is no proven advantage of the FoundationOne Liquid CDx over targeted EGFR mutation testing or small targeted panels for this indication.

Aetna considers testing to detect PD-L1 expression (e.g., Ventana PD-L1 (SP263) Assay) medically necessary for persons with urothelial carcinoma who are being considered for treatment with durvalumab (Imfinzi).

Aetna considers testing to detect PD-L1 expression (e.g., Ventana PD-L1 (SP142) Assay) medically necessary for persons with triple-negative breast carcinoma (TNBC), non-small cell lung cancer and urothelial carcinoma who are being considered for treatment with atezolizumab (Tecentriq).

Aetna considers an testing for platelet-derived growth factor receptor-beta (PDGFRß) gene rearrangements (e.g., PDGFRB FISH) medically necessary for persons with chronic myelomonocytic leukemia and myelodysplastic syndrome/myeloproliferative disease being considered for treatment with imatinib mesylate (Gleevec).

Aetna considers c-KIT testing medically necessary for persons with gastrointestinal stromal tumors (GIST) (e.g., Dako c-KIT pharmDx) or aggressive systemic mastocytosis (KIT D816V Mutation Detection by PCR) being considered for treatment with imatinib mesylate (Gleevec).
Aetna considers an FTL3 mutation assay (e.g., LeukoStrat CDx FLT Mutation Assay) medically necessary for persons with acute myeloid leukemia (AML) being considered for treatment with midostaurin (Rydapt), gilternib (Xospata) or sorafenib (Nexavar).

Aetna considers measurement of microsatellite instability (MSI-H) and mismatch repair deficiency (DMMR) medically necessary for persons with metastatic colorectal cancer being considered for treatment with ipilimumab (Yervoy).

Aetna considers measurement of microsatellite instability (MSI-H) and mismatch repair deficiency (DMMR) medically necessary for persons with metastatic colorectal cancer being considered for treatment with nivolumab (Opdivo).

Aetna considers measurement of microsatellite instability (MSI-H) and mismatch repair deficiency (DMMR) medically necessary for persons with unresectable or metastatic solid tumors being considered for treatment with pembrolizumab (Keytruda).

Aetna considers testing to detect PD-L1 expression (e.g., PD-L1 IHC 22C3 pharmDx) medically necessary for persons with non-small cell lung cancer being considered for treatment with pembrolizumab (Keytruda).

Aetna considers FoundationOne CDx testing medically necessary to identify advanced cancer in members with solid tumors that are tumor mutational burden-high (TMB greater than or equal to 10 mutations per megabase) and appropriate for treatment with pembrolizumab (Keytruda).

Aetna considers an isocitrate dehydrogenase-1 (IDH1) mutation or isocitrate dehydrogenase-2 (IDH2) mutation (e.g., Abbott Real Time IDH1 and IDH2) medically necessary for persons with acute myeloid leukemia (AML) being considered for treatment with enasidenib mesylate (Idhifa) or ivosidenib (Tibsovo).

Aetna considers del(17p)/TP53 mutation testing (e.g., Vysis CLL FISH Probe Kit) medically necessary for persons with chronic lymphocytic leukemia/small lymphocytic lymphoma being considered for treatment with venetoclax (Venclexta).

Aetna considers BCR/ABL mutation testing (e.g., MRDx BCR-ABL Test) medically necessary for persons with chronic myeloid leukemia being considered for treatment with nilotinib (Tasigna).
Aetna considers FGFR2 and FGFR3 mutation testing medically necessary for persons with urothelial carcinoma being considered for treatment with erdafitinib (Balversa).

Aetna considers FGFR2 mutation testing (e.g., FoundationOne CDx) medically necessary for persons with cholangiocarcinoma being considered for treatment with pemigatinib (Pemazyre).

Aetna considers PIK3CA mutation testing (e.g., therascreen PIK3CA RGQ PCR Kit) medically necessary for persons with breast cancer being considered for treatment with alpelizib (Piqray). Aetna considers FoundationOne CDx testing panel not medically necessary for assessing candidacy of persons with breast cancer for treatment with alpelisib (Piqray), because there is no proven advantage of the FoundationOne CDx panel over targeted PIK3CA mutation testing for this indication.

Aetna considers mesenchymal-epithelial transition (MET) exon 14 skipping mutation testing (e.g., FoundationOne CDx) medically necessary for persons with advanced, recurrent or metastatic non-small cell lung cancer being considered for treatment with the kinase inhibitor capmatinib (Tabrecta).

Aetna considers EZH2 mutation testing medically necessary for persons with follicular lymphoma being considered for treatment with tazemetostat (Tazverik).

Aetna considers RET fusion testing (e.g., Oncomine Dx Target Test) medically necessary for persons with non-small cell lung cancer (NSCLC) being considered for treatment with pralsetinib (Gavreto).

Aetna considers ERBB2 (HER2) amplification testing medically necessary for persons with breast, colorectal, esophageal, gastric, gastroesophageal junction, non-small cell lung, salivary gland, and uterine serous cancer being considered for treatment with trastuzumab (Herceptin and biosimilars), ado-trastuzumab emtansine (Kadcyla), or pertuzumab (Perjeta). See CPB 0313 - Trastuzumab, Ado-Trastuzumab, Trastuzumab and Hyaluronidase-oysk, and Pertuzumab.

Aetna considers the following tests experimental and investigational:

- multi-gene pharmacogenetics panels (i.e., diagnostic tests to identify specific genetic variations that may be linked to reduced/enhanced metabolism and/or severe side effects of multiple classes of drugs) experimental and investigational because the clinical value of this type of genetic testing has not been established. These tests include, but are not limited to the following:
Genecept Assay, GeneSightRx, GeneSight ADHD, GeneSight Analgesic, GeneSight MTHFR and GeneSight Psychotropic testing, Millennium PGT, PersonaGene Genetic Panels, Proove Profiles, rxSEEK Epilepsy Drug Metabolism, OneOme RightMed Pharmacogenomic Test, INFINITI Neural Response Panel, and IDgenetix, and Psych HealthPGx Panel by RPRD Diagnostics.

A. Aetna considers the UGT1A1 molecular assay (a screening test for determining the proper dosage of irinotecan for persons with colorectal cancer or other types of cancer (e.g., non-small-cell lung cancer) experimental and investigational because its clinical value has not been established.

B. Aetna considers genotyping for VKORC1 polymorphism (diagnostic tests to identify specific genetic variations that may be linked to reduced/enhanced effect or severe side effects of drugs metabolized by the vitamin K epoxide reductase complex subunit 1 gene including warfarin) experimental and investigational because the clinical value of this type of genetic testing has not been established.

C. Aetna considers genotyping for apolipoprotein E (Apo E) for determining therapeutic response to lipid-lowering medications experimental and investigational because its clinical value has not been established.

D. Aetna considers genotyping for methylenetetrahydrofolate reductase (MTHFR) for determining therapeutic response to antifolate chemotherapy and for guiding antidepressant therapy experimental and investigational because its clinical value has not been established.

E. Aetna considers measurement of thromboxane metabolites in urine (e.g., AspirinWorks) to evaluate aspirin resistance experimental and investigational because its clinical value has not been established.

F. Aetna considers genetic testing for the rs3798220 allele experimental and investigational for selecting persons for chronic aspirin therapy or other indications because its clinical value has not been established.

G. Aetna considers laboratory testing to allow area under the curve (AUC)-targeted 5-fluorouracil dosing (e.g., Myriad Genetics’ OnDose) experimental and investigational because its clinical value has not been established.
H. Aetna considers testing for genetic polymorphisms of dihydropyrimidine dehydrogenase and thymidylate synthase to predict 5-fluorouracil toxicity experimental and investigational because the clinical value of such testing has not been established.

I. Aetna considers IL28B polymorphism genotyping for interferon therapy for hepatitis C experimental and investigational because its clinical value has not been established.

J. Aetna considers platelet reactivity/function testing (VerifyNow P2Y12 Assay, Ultegra System Rapid Platelet Function Assay-ASA) for individuals who have undergone percutaneous coronary intervention experimental and investigational because its clinical value has not been established.

K. Aetna considers methotrexate polyglutamates (Avise PG test) experimental and investigational for evaluating response to methotrexate therapy because there is no clinical evidence to support the use of the Avise PG test for evaluating response to methotrexate therapy in rheumatoid arthritis or other conditions.

L. Aetna considers beta adrenergic receptor genotyping experimental and investigational for evaluating persons with treatment resistant asthma and for all other indications.

M. Aetna considers genotyping for A1555G before prescribing aminoglycosides experimental and investigational.

N. Aetna considers Amerigene PGT pharmacogenetic testing panels experimental and investigational because their clinical value has not been established.

O. Aetna considers Aegis Drug-Drug Interaction Test experimental and investigational because its clinical value has not been established.

P. Aetna considers genotyping of interferon-lambda 3 (IFNL3) experimental and investigational for prediction of virological response to pegylated-interferon-alpha and ribavirin combination therapy.
Q. Aetna considers PGxOnePlus genetic testing experimental and investigational for anxiety and gastroesophageal reflux disease because its clinical value has not been established.

R. Aetna considers UrSure tenofovir quantification test experimental and investigational for adherence monitoring to pre-exposure prophylaxis because its clinical value has not been established.

S. Aetna considers ComplyRX urine test by Claro Labs experimental and investigational for drug monitoring because its clinical value has not been established.

T. Aetna considers OncolyticAssuranceRX by Firstox experimental and investigational for adherence monitoring to oral anticancer medications because its clinical value has not been established.

U. Aetna considers Snapshot Oral Fluid Compliance by Ethos experimental and investigational for adherence monitoring to prescription drugs because its clinical value has not been established.

For HER-2/neu testing, see CPB 0313 - Trastuzumab (Herceptin), Ado-Trastuzumab (Kadcyla), Pertuzumab (Perjeta) and Trastuzumab and Hyaluronidase-oysk (Herceptin Hylacta).

For HER-2/neu testing, see CPB 0313 - Trastuzumab (Herceptin), Ado-Trastuzumab (Kadcyla), Pertuzumab (Perjeta) and Trastuzumab and Hyaluronidase-oysk (Herceptin Hylacta).

See also

- CPB 0140 - Genetic Testing,

- CPB 0227 - BRCA Testing, Prophylactic Mastectomy, and Prophylactic Oophorectomy,
Background

Adverse drug reactions (ADRs) are responsible for many debilitating side effects and are a major cause of death following drug therapy. It is now clear that a significant portion of these ADRs as well as therapeutic failures are caused by genetic polymorphism and genetically based inter-individual differences in drug absorption, disposition, excretion or metabolism.

Pharmacogenomics testing is laboratory testing which has the potential to determine how an individual’s genetic factors may affect the safety and effectiveness of that individual’s response to a specific medication. The goal of pharmacogenomics testing is to reduce the incidence of adverse medication reactions while improving an individual’s positive response to the medication. Additionally, some tests may help provide information on how well a specific treatment may work for an individual.

Genotyping for Cytochrome P450 Polymorphisms

Cytochrome P450 enzymes are a group of enzymes that account for approximately 75 percent of drug metabolism in the human body. Enzymes encoded by the P450 genes (eg, CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP3A5 etc.) are found primarily in the liver. The action of the P450 enzymes affects the blood levels of many drugs. Genotyping for cytochrome P450 has been proposed for possible use in medical management of drug therapies including, but not limited to, antidepressants, antiepileptic, antipsychotics, barbiturates, clopidogrel, opioid analgesics, proton pump inhibitors, tamoxifen or warfarin.

Recent advances in molecular biology have improved the understanding of genetic factors underlying many ADRs. Until recently, investigations in this field have generally centered on gene coding for drug-metabolizing enzymes. Inactivating mutations have
been found in gene coding for enzymes belonging to the cytochrome P450 (CYP) system, which is important in the hepatic metabolism of many drugs. Individuals with a lack of functional activity in these enzymes should be treated with very low doses of drugs metabolized by the same enzyme to avoid excessive drug levels and thus toxic effects. In recent years, research has been focused on gene coding for drug targets. As a result, most studies have been performed on single genes known to be or assumed to be functionally related to a given ADR. An alternative method is testing for complex single nucleotide polymorphisms that may be associated with ADRs, although the functional relationship between them may be completely unknown. As a consequence of influence from non-genetic factors in the development of ADRs, the association between a specific genotype and an ADR will always be less than 100%. Thus, there is a need for well-designed clinical trials to ascertain the extent of environmental influences on the ADRs for which a genetic basis has been implicated (Guzey and Spigset, 2004). This is in agreement with the observation of Pirmohamed and Park (2003) who stated that “ADRs are common and many are suggested to have a genetic predisposition. There has been intense research in the role of CYP enzyme gene polymorphisms in the cause of ADRs. The major impact to date of polymorphic CYP expression has been on pre-clinical drug development. The direct clinical impact of CYP polymorphisms on prediction of ADRs, however, has been limited mainly because published reports have been small and retrospective and their findings conflicting. Moreover, the clinical- and cost-effectiveness of pre-prescription genotyping for CYP polymorphisms has not been established. More investigation is needed before prospective CYP genotyping can become routine clinical practice”.

Kirchheiner and Brockmoller (2005) stated that the genetic coding for the CYP enzyme 2C9 (CYP2C9) carries many inherited polymorphisms. Those coding for R144C (*2) and I359L (*3) amino acid substitutions have both significant functional effects and appreciable high population frequencies, and their in vivo consequences have been examined in humans in relation to drug metabolism -- pharmacokinetics, drug responses as well as outcomes of clinical trials in subjects with different CYP2C9 genotypes. Tentative estimates of how CYP2C9 genotyping might be applied to dose adjustments in clinical therapy were based on dose-related pharmacokinetic parameters such as clearance or trough drug concentrations. Mean clearances in homozygous carriers of the *3 allele were below 25% of that of the wild type for S-warfarin, tolbutamide, glipizide, celecoxib, and fluvastatin. In the more frequent heterozygous carriers (genotype *1/*3), the clearances were between 40 and 75%. In these cases in which individual dosages are derived from clinical drug effects, such as for oral anticoagulants, the pharmacogenetics-based dose adjustments showed a good correlation with the genotype-specific empirically derived doses. In addition to its role in pharmacokinetics, CYP2C9 contributes to the metabolism of fatty acids, prostanoids,
and steroid hormones, and it may catalyze potentially toxic bioactivation reactions. However, the current understanding of the role of CYP2C9 in biotransformation of endogenous signaling molecules and in drug toxicity is relatively meager. These investigators concluded that the concept of therapy based on genotyping for CYP polymorphisms has not been assessed in prospective, randomized, controlled studies in which one group is dosed according to genotype while another group is dosed in a usual manner. It is unlikely that CYP2C9 genotyping will become routine practice unless its clinical value is supported by such rigorous assessments.

The following is a list of some CYP2C9-metabolized drugs with narrow therapeutic ratios (drugs with a narrow difference between therapeutic and toxic concentrations) with serious toxic effects:

- Angiotensin II blockers (e.g., irbesartan, losartan)
- NSAIDS (e.g., diclofenac, ibuprofen, naproxen, piroxicam)
- Oral hypoglycemic agents (e.g., glipizide, tolbutamide)
- Sulfonylureas (e.g., amitriptyline, celecoxib, fluoxetine, fluvastatin, glipizide, rosiglitazone, tamoxifen, tolbutamide, S-warfarin).

Diagnostic genotyping tests for some CYP enzymes are now available commercially. The AmpliChip (Roche Diagnostics, Basel, Switzerland), cleared by the United States Food and Drug Administration (FDA) through the 510(k) process, is a microarray consisting of many DNA sequences complementary to 2 CYP genes applied in microscopic quantities at ordered locations on a solid surface (chip). The AmpliChip tests the DNA from a patient’s leukocytes collected in a standard anti-coagulated blood sample for 29 polymorphisms and mutations for the CYP2D6 gene and 2 polymorphisms for the CYP2C19 gene. CYP2D6 metabolizes about 25% of all clinically used medications (e.g., antiarrhythmics, antidepressants, beta-blockers, dextromethorphan and morphine derivatives), while CYP2C19 metabolizes several important types of drugs including anti-epileptics and proton-pump inhibitors. The AmpliChip was cleared for marketing for CYP2D6 testing in December 2004, and for CYP2C19 testing in January 2005.

The AmpliChip is marketed for use in screening of patients who are to be treated with drugs metabolized by the CYP system that have a narrow therapeutic ratio with serious toxic effects.

The following is a list of CYP2D6-metabolized drugs with narrow therapeutic ratios (not an all inclusive list):
• Anti-depressants (e.g., amitryptiline, clomipramine, desipramine, imipramine, paroxetine)
• Anti-psychotics (e.g., chlorpromazine, haloperidol, perphenazine, risperidone, thioizadine)
• Beta blockers (e.g., carvediol, propafenone, timolol).

The following is a list of CYP2C19-metabolized drugs with narrow therapeutic ratios (not an all inclusive list):

• Anti-epileptics (e.g., amitryptiline, clomipramine, cyclophosphamide, diazepam, imipramine, moclobemide, phenytoin, phenobarbitone, primidone, R-warfarin)
• Proton pump inhibitors (e.g., lansoprazole, omeprazole, pantoprazole)
• Others (e.g., clopidogrel).

Footnotes for list These lists are based on information excerpted from the “Cytochrome P450 Drug Interaction Table”, Indiana University School of Medicine, 2009. Randomized controlled trials are needed to ascertain if the AmpliChip will lower the incidence of ADRs by detecting patients with CYP2D6 and CYP2C19 mutations. The effectiveness of the AmpliChip in reducing toxic effects and improving health outcomes would need to be compared with standard methods of therapeutic drug monitoring (e.g., by monitoring clinical response or by measuring serum drug concentrations). Moreover, it is still unclear that the AmpliChip would eliminate the need for simultaneous use of other methods of therapeutic drug monitoring (including serum measurements) because factors other than genetic polymorphisms such as those described earlier have a significant effect on drug pharmacokinetics and pharmacodynamics.

Juran and colleagues (2006) stated that “[e]ven though the AmpliChip CYP450 has been approved by the FDA, its practical clinical utility has not yet been determined, and there is a paucity of data related to gastrointestinal and liver diseases. An understanding of the principles and opportunities provided by this new category of diagnostic test is key before planning the necessary studies to evaluate the usefulness of AmpliChip CYP450 in gastroenterologic clinical practice”.

A special report (BlueCross BlueShield Technology Evaluation Center, 2004) on genotyping for CYP polymorphisms to determine drug-metabolizer status stated that diagnostic tests to identify specific polymorphisms that may be linked to increased/reduced effect or serious adverse effects are now available. Whether such testing, and for which drugs, improves patient outcomes is not yet known. While genotyping for the CYP enzymes would only need to be performed once per patient
and the results could be used to consider other drugs metabolized by the same enzymes, whether genotyping is clinically useful would need to be determined for each drug. Even drugs of the same class may variably rely on specific CYP enzymes. For example, the plasma level of the selective serotonin reuptake inhibitor (SSRI) fluoxetine is significantly affected by CYPP2D6 polymorphisms, whereas the SSRI sertraline appears to be little affected and may depend more upon CYP2C19 for metabolism. The report stated that while the potential of pharmacogenetic studies is intriguing for many clinical applications, it is still unclear which are most likely to provide clinical benefit in the near future.

Ingelman-Sundberg (2005) stated that the polymorphism of CYP2D6 significantly affects the pharmacokinetics of approximately half of the drugs in clinical use, which are CYP2D6 substrates. The consequences of the polymorphism at ordinary drug doses can be either ADRs or no drug response. Predictive CYP2D6 genotyping is estimated to be beneficial for treatment of about 30 to 40% of CYP2D6 drug substrates representing about 7 to 10% of all clinically used drugs, although prospective clinical studies are needed to determine the exact benefit of drug selection and dosage based on the CYP2D6 genotype. Furthermore, Sanderson et al (2005) assessed the strength and quality of existing evidence about CYP2C9 gene variants and clinical outcomes in warfarin-treated patients in a meta-analysis (11 studies with a total of 2,775 patients). These investigators concluded that patients with CYP2C9*2 and CYP2C9*3 alleles have lower mean daily warfarin doses and a greater risk of bleeding. The authors also stated that while testing for gene variants could potentially alter clinical management in patients commencing warfarin, evidence for the clinical utility and cost-effectiveness of genotyping is necessary before routine testing can be recommended.

In a review article on “Drug Metabolism and Variability among Patients in Drug Response” published in the New England Journal of Medicine, Wilkinson (2005) stated that “there have not yet been prospective clinical trials showing that knowledge of a patient’s genotypic profile before prescribing drugs either increases drug efficacy, prevents or reduces adverse drug reactions, or lower the overall costs of therapy and associate sequelae .... For now, however, the individual patient is probably best served by an alert physician aware of the possibility that a genetic polymorphism in drug metabolism may be a potential factor in unexpected drug response”.

In a multi-center 6-week study, Fux et al (2005) examined the impact of CYP2D6 polymorphism on the tolerability of metoprolol in a primary care setting. The adverse effects studied comprised effects related to the central nervous system, cardiovascular effects, and sexual dysfunction. The dosage of metoprolol was determined on an individual basis and could be adjusted on clinical grounds. The indication for treatment
was hypertension in about 90 % of cases. CYP2D6 genotyping covered alleles *3 to *10 and *41 and the duplications. Possible ADRs of metoprolol were systematically assessed over the study period using standardized rating scales and questionnaires. The final study population comprised 121 evaluable patients: 5 ultrarapid metabolizers (UMs) (4.1 %), 91 extensive metabolizers (EMs) (75 %), 21 intermediate metabolizers (IMs) (17 %), and 4 poor metabolizers (PMs) (3.3 %). Plasma metoprolol concentrations normalized for the daily dose and metoprolol/alpha-hydroxymetoprolol ratios at steady state were markedly influenced by CYP2D6 genotype and displayed a gene-dose effect. The median of the dose-normalized metoprolol concentration was 0.0088 ng/ml, 0.047 ng/ml, 0.34 ng/ml, and 1.34 ng/ml among UMs, EMs, IMs, and PMs, respectively (p < 0.0001). There was no significant association between CYP2D6 genotype-derived phenotype (EMs and UMs combined versus PMs and IMs combined) and ADRs during treatment with metoprolol. There was a tendency toward a more frequent occurrence of cold extremities in the PM plus IM group as compared with the EM plus UM group (16.0 % versus 4.2 %, p = 0.056; relative risk, 3.8 [95 % confidence interval (CI): 1.03 to 14.3]). These investigators concluded that CYP2D6 genotype-derived phenotype was not significantly associated with a propensity for ADRs to develop during treatment with metoprolol. However, the results concerning tolerability of metoprolol in PMs were inconclusive because of the small number of PMs enrolled.

The mechanisms of variable response to tamoxifen have been the subject of much scrutiny in the published literature. Early studies attempting to link a clinical response to tamoxifen therapy with plasma tamoxifen concentrations reported no statistically significant differences in outcomes between women who received 20 mg of tamoxifen daily and those who received 40 mg of tamoxifen daily, even though women in the 40 mg tamoxifen group had higher plasma tamoxifen concentrations than those in the 20 mg tamoxifen group. These results have been reported as evidence that plasma tamoxifen concentration is not a predictor of clinical outcome. Because there is evidence that tamoxifen is converted to anti-estrogenic metabolites, one hypothesis is that altered patterns of metabolism of tamoxifen might contribute to inter-individual variability in effects (Jin et al, 2005). Plasma concentrations of the active tamoxifen metabolite endoxifen are associated with the cytochrome P450 (CYP) 2D6 genotype.

Goetz et al (2005) stated that polymorphisms in tamoxifen metabolizing genes affect the plasma concentration of tamoxifen metabolites, but their effect on clinical outcome is unknown. These investigators determined cytochrome P450 (CYP)2D6 (*4 and *6) and CYP3A5 (*3) genotype from paraffin-embedded tumor samples and buccal cells (living patients) in tamoxifen-treated women enrolled onto a North Central Cancer Treatment Group adjuvant breast cancer trial. The relationship between genotype and disease outcome was determined using the log-rank test and Cox proportional hazards
modeling. Paraffin blocks were obtained from 223 of 256 eligible patients, and buccal cells were obtained from 17 living women. CYP2D6 (*4 and *6) and CYP3A5 (*3) genotypes were determined from 190, 194, and 205 patient samples and in 17 living women. The concordance rate between buccal and tumor genotype was 100%.

Women with the CYP2D6 *4/*4 genotype had worse relapse-free time (RF-time; \( p = 0.023 \)) and disease-free survival (DFS; \( p = 0.012 \)), but not overall survival (\( p = 0.169 \)) and did not experience moderate to severe hot flashes relative to women heterozygous or homozygous for the wild-type allele. In the multi-variate analysis, women with the CYP2D6 *4/*4 genotype still tended to have worse RFS (hazard ratio [HR], 1.85; \( p = 0.176 \)) and DFS (HR, 1.86; \( p = 0.089 \)). The CYP3A5*3 variant was not associated with any of these clinical outcomes. The authors concluded that in tamoxifen-treated patients, women with the CYP2D6 *4/*4 genotype tend to have a higher risk of disease relapse and a lower incidence of hot flashes, which is consistent with their previous observation that CYP2D6 is responsible for the metabolic activation of tamoxifen to endoxifen. They noted that "[t]hese findings have the potential to improve the ability of physicians to select the optimal hormonal therapy for the treatment of ER-positive breast cancer. Further studies are needed in women receiving tamoxifen to fully define the effect of CYP2D6 genetic polymorphisms and medications that inhibit CYP2D6 on tamoxifen response."

An assessment of CYP2D6 pharmacogenomics of tamoxifen treatment conducted by the BlueCross BlueShield Association Technology Evaluation Center (2008) evaluated the evidence for CYP2D6 genotyping and tamoxifen treatment efficacy. The hypothesis examined by the assessment is that CYP2D6 poor metabolizers, whether by genotype or by co-administration of CYP2D6 inhibitory medication, have reduced tamoxifen metabolism and lower endoxifen levels compared to better metabolizers, and as a result have poorer clinical outcomes. The reviewers stated that this hypothesis is based on the assumption, not yet supported by evidence, that some level of endoxifen is necessary for tamoxifen efficacy and that this level is not achieved in genotypic and functional CYP2D6 poor metabolizers and possibly not in some intermediate metabolizers. However, the reviewers found no scientific evidence for a significant association between endoxifen and clinical outcomes. In addition, they reported several limitations on the association of genotype with clinical outcomes. The reviewers stated, "Because tamoxifen metabolism is complex and CYP2D6 does not appear to account for all variability in endoxifen levels, it is conceivable that polymorphisms in other tamoxifen metabolic pathway enzymes may affect active metabolite levels, and direct measurement of the metabolite(s) itself may be the better predictor of benefit from tamoxifen treatment. However, since it takes 8 weeks for tamoxifen metabolites to reach steady-state concentrations, measuring metabolite levels is not practical for clinical applications outside of a retrospective study." Furthermore, the reviewers noted
that multiple enzyme genotypes may be needed to confidently predict tamoxifen versus aromatase inhibitors treatment benefit; however, there are little data at present to recommend any genotype combinations. The assessment concluded, "There is insufficient evidence to permit conclusions regarding the use of CYP2D6 genotyping for directing endocrine therapy regimen selection for women at high risk for or with breast cancer."

Visvanathan et al (2009) updated the 2002 American Society of Clinical Oncology guideline on pharmacologic interventions for breast cancer risk reduction. The cytochrome P450 2D6 gene (CYP2D6) encodes the enzyme responsible for catalyzing the conversion of tamoxifen to endoxifen, an active metabolite of tamoxifen. Functional allelic variants (*4 most common in whites and *10 most common in Asians), have been identified in approximately 7% of the population. Lower levels of endoxifen have been observed in women taking tamoxifen who are heterozygous and homozygotes for variant alleles in CYP2D6 in a dose-dependent manner, or in women treated with concomitant medications that block CYP2D6, including certain selective serotonin reuptake inhibitors such as paroxetine. Further, in a small nested case-control study of women who took part in the Italian prevention trial, a higher prevalence of the CYP2D6 *4/*4 phenotype was observed among women with breast cancer who took tamoxifen compared with controls. The authors concluded that confirmation of these results in larger studies is needed. Given the limited evidence, CYP2D6 testing is currently not recommended in the preventive setting.

The BlueCross BlueShield Association's technology assessment on CYP2D6 pharmacogenomics of tamoxifen treatment (2011) stated that studies showing no evidence of association between CYP2D6 genotype and either tamoxifen- or aromatase inhibitor-treated patient outcomes has suggested that using the results of CYP2D6 genetic testing to influence decisions about tamoxifen treatment is not currently warranted.

A BlueCross BlueShield Association Technology Evaluation Center (TEC, 2013) assessment of CYP2D6 pharmacogenetics of tamoxifen treatment concluded that CYP2D6 genotyping does not meet the TEC criteria for directing endocrine therapy regimen selection for women at high risk for primary breast cancer or breast cancer recurrence. The assessment stated that there are several limitations to the overall body of evidence, but the largest, most well-designed studies do not support clinical validity of the test. In the absence of evidence for clinical validity, evidence to support clinical utility is lacking.
Regan et al (2012) noted that adjuvant tamoxifen therapy is effective for post-menopausal women with endocrine-responsive breast cancer. Cytochrome P450 2D6 (CYP2D6) enzyme metabolizes tamoxifen to clinically active metabolites, and CYP2D6 polymorphisms may adversely affect tamoxifen efficacy. In this study, these researchers investigated the clinical relevance of CYP2D6 polymorphisms. They obtained tumor tissues and isolated DNA from 4,861 of 8,010 post-menopausal women with hormone receptor-positive breast cancer who enrolled in the randomized, phase III double-blind Breast International Group (BIG) 1-98 trial between March 1998 and May 2003 and received tamoxifen and/or letrozole treatment. Extracted DNA was used for genotyping nine CYP2D6 single-nucleotide polymorphisms using polymerase chain reaction-based methods. Genotype combinations were used to categorize CYP2D6 metabolism phenotypes as poor, intermediate, and extensive metabolizers (PM, IM, and EM, respectively; n = 4,393 patients). Associations of CYP2D6 metabolism phenotypes with breast cancer-free interval (referred to as recurrence) and treatment-induced hot flushes according to randomized endocrine treatment and previous chemotherapy were assessed. Cox proportional hazards models were used to calculate hazard ratios (HRs) and 95 % confidence intervals (CIs). All statistical tests were two-sided. No association between CYP2D6 metabolism phenotypes and breast cancer-free interval was observed among patients who received tamoxifen monotherapy without previous chemotherapy (p = 0.35). PM or IM phenotype had a non-statistically significantly reduced risk of breast cancer recurrence compared with EM phenotype (PM or IM versus EM, HR of recurrence = 0.86, 95 % CI: 0.60 to 1.24). CYP2D6 metabolism phenotype was associated with tamoxifen-induced hot flushes (p = 0.020). Both PM and IM phenotypes had an increased risk of tamoxifen-induced hot flushes compared with EM phenotype (PM versus EM, HR of hot flushes = 1.24, 95 % CI: 0.96 to 1.59; IM versus EM, HR of hot flushes = 1.23, 95 % CI: 1.05 to 1.43). The authors concluded that CYP2D6 phenotypes of reduced enzyme activity were not associated with worse disease control but were associated with increased hot flushes, contrary to the hypothesis. The results of this study do not support using the presence or absence of hot flushes or the pharmacogenetic testing of CYP2D6 to determine whether to treat post-menopausal breast cancer patients with tamoxifen.

Rae et al (2012) stated that adjuvant tamoxifen therapy substantially decreases the risk of recurrence and mortality in women with hormone (estrogen and/or progesterone) receptor-positive breast cancer. Previous studies have suggested that metabolic conversion of tamoxifen to endoxifen by cytochrome P450 2D6 (CYP2D6) is required for patient benefit from tamoxifen therapy. Tumor specimens from a subset of post-menopausal patients with hormone receptor-positive early-stage (stages I, II, and IIIA) breast cancer, who were enrolled in the randomized double-blind Arimidex, Tamoxifen, Alone or in Combination (ATAC) clinical trial, were genotyped for variants in CYP2D6 (n...
= 1,203 patients: anastrozole [trade name: Arimidex] group, n = 615 patients; tamoxifen group, n = 588 patients) and UDP-glucuronosyltransferase-2B7 (UGT2B7), whose gene product inactivates endoxifen (n = 1,209 patients; anastrozole group, n = 606 patients; tamoxifen group, n = 603 patients). Genotyping was performed using polymerase chain reaction-based TaqMan assays. Based on the genotypes for CYP2D6, patients were classified as poor metabolizer (PM), intermediate metabolizer (IM), or extensive metabolizer (EM) phenotypes. These investigators evaluated the association of CYP2D6 and UGT2B7 genotype with distant recurrence (primary endpoint) and any recurrence (secondary endpoint) by estimating the hazard ratios (HRs) and corresponding 95% CIs using Cox proportional hazards models. All statistical tests were 2-sided. After a median follow-up of 10 years, no statistically significant associations were observed between CYP2D6 genotype and recurrence in tamoxifen-treated patients (PM versus EM: HR for distant recurrence = 1.25, 95% CI: 0.55 to 3.15, p = 0.64; HR for any recurrence = 0.99, 95% CI: 0.48 to 2.08, p = 0.99). A near-null association was observed between UGT2B7 genotype and recurrence in tamoxifen-treated patients. No associations were observed between CYP2D6 and UGT2B7 genotypes and recurrence in anastrozole-treated patients. The authors concluded that these findings do not support the hypothesis that CYP2D6 genotype predicts clinical benefit of adjuvant tamoxifen treatment among post-menopausal breast cancer patients.

In a review on pharmacogenomics and individualized drug therapy, Eichelbaum et al (2006) stated that "[t]here is also a growing list of genetic polymorphisms in drug targets that have been shown to influence drug response. A major limitation that has heretofore moderated the use of pharmacogenetic testing in the clinical setting is the lack of prospective clinical trials demonstrating that such testing can improve the benefit/risk ratio of drug therapy". Furthermore, Humphries and Hingorani (2006) noted that the full potential of the field of pharmacogenetics will only be realized with much further work.

An assessment of CYP450 genetic testing by the Canadian Coordinating Office for Health Technology Assessment (Palylyk-Colwell, 2006) concluded: "No published studies show that patient outcomes can be predicted or altered by knowledge of DME status in the absence of other confounding variables. Prospective studies are needed to assess the benefits and potential risks of this technology in guiding drug selection and dose adjustment."

Since their introduction in the late 1980s, SSRIs such as citalopram, fluoxetine, paroxetine, and sertraline have become the most commonly prescribed class of drugs for treating depression. However, the likelihood that a patient will experience relief from all symptoms of depression after 1 year of treatment is approximately 40%, and
adverse events cause 12 to 15% of patients who start treatment to stop taking the
drug. Following the recent FDA approval of a test to predict differences in the CYP450
gene, physicians and patients must decide if using such tests to choose a type or dose
of an SSRI might improve the patient's response to treatment.

Kirchheiner and colleagues (2003) noted that antidepressants are characterized by a
high rate of drug failure. There is evidence that genetic factors are contributing to the
inter-individual variability in response to these medications. Genetic polymorphisms in
drug metabolizing enzymes are well established and have significant effects on oral
clearances or elimination half-lives of antidepressants. These differences can be
compensated by adapting the individual dose to genotype in addition to other factors
such as age, gender, weight, drug interactions, diseases (cardiovascular, hepatic, renal
and respiratory) as well as environmental influences on drug metabolism (e.g., diet and
smoking). Genetic variability is found in molecular structures of antidepressant
effects. Furthermore, Kirchheiner et al (2003) noted that studies on response of
antidepressants have revealed influences of polymorphisms in neurotransmitter
receptors and transporters altering sensitivity of patients to treatment with
antidepressants; however, results were often contradictory.

The Agency for Healthcare Research and Quality (AHRQ, 2006) released an evidence
report that found there is insufficient evidence to determine if current gene-based tests
intended to personalize the dose of SSRIs improve patient outcomes or aid physicians
or patients in making treatment decisions. The available studies indicated that the tests
are largely accurate at evaluating differences in genes belonging to the CYP450 family
that affect the rate at which a person metabolizes SSRIs. However, additional well-
designed studies are needed to determine the usefulness of test results in the clinical
setting. This report is the first step in the 2-step process of Centers for Disease Control
and Prevention's Evaluation of Genomic Applications in Practice and Prevention (EGAPP)
pilot project to evaluate and make recommendations regarding the use of gene-based
tests.

The Evaluation of Genomic Applications in Practice and Prevention Working Group
(EGAPP, 2007) found insufficient evidence to support a recommendation for or against
use of cytochrome P450 (CYP450) testing in adults beginning SSRI treatment for non-
psychotic depression. In the absence of supporting evidence, and with consideration of
other contextual issues, EGAPP discourages use of CYP450 testing for patients
beginning SSRI treatment until further clinical trials are completed. EGAPP found that
use of genetic testing for CYP450 polymorphisms and impact on physician decision-
making with regard to use of SSRIs is not known. EGAPP noted that, in the absence of
evidence supporting clinical utility, widespread use of CYP450 genetic testing is
potentially costly and may not lead to changes in treatment that improve patient outcomes.

The eradication rates of *Helicobacter pylori* by triple therapy consisting of 1 proton pump inhibitor (PPI) and 2 antimicrobial agents are mainly influenced by bacterial susceptibility to anti-microbial agents and the magnitude of acid inhibition during treatment with a PPI (e.g., omeprazole, lansoprazole, rabeprazole). Tailored therapy using CYP2C19 pharmacogenomics with a PPI has been proposed as a method to help improve the efficacy of *H. pylori* eradication rates.

A review on the use of pharmacogenomics-based treatment of *H. pylori* infection conducted by the BlueCross BlueShield Association Technology Evaluation Center (2008) examined the scientific evidence of a pharmacogenomics-based treatment regimen for the eradication of *H. pylori*. The reviewers found 1 randomized, controlled study that met their inclusion criteria. The reviewers reported that this study found higher eradication rates after first-line treatment for the pharmacogenomics group compared with the standard treatment group, however, because of numerous variations in treatment protocol within the pharmacogenomics group, it was not possible to determine whether the improvement resulted from the tailored PPI dosages according to CYP2C19 genetic status, or if it was due to other variations in the treatment protocol unrelated to CYP2C19 status. Furthermore, the review noted that it was possible other clinical factors, such as clarithromycin resistance, or other treatment factors, such as length of antibiotic treatment, influenced eradication rates. In addition, the study was performed in a Japanese population and did not employ a diagnostic approach or a treatment regimen that is standard care in the United States. The review concluded, "The scientific evidence does not permit conclusions on whether the use of a pharmacogenomics-based treatment regimen for *H. pylori* improves eradication rates."

Clopidogrel bisulfate (Plavix) (Bristol-Myers Squibb/Sanofi Pharmaceuticals, Bridgewater, NJ) is an orally administered antiplatelet drug that is used to prevent blood clots that could lead to heart attacks or strokes in individuals with established cardiovascular atherosclerotic disease. The degree of platelet inhibition seen following use of the second-generation thienopyridine clopidogrel varies from patient to patient in a normal or bell-shaped distribution. The variability in non-response is such that, when laboratory measurements of platelet aggregation are performed, 4 to 30 % of patients treated with clopidogrel do not have an adequate anti-platelet response.

Studies have linked reduced response to clopidogrel to variants in the gene CYP2C19. Mega et al (2009) reported that in healthy subjects who were treated with clopidogrel, carriers of at least one CYP2C19 reduced-function allele had a relative
reduction of 32.4% in plasma exposure to the active metabolite of clopidogrel, as compared with non-carriers. The CYP2C19*1 allele corresponds to fully functional metabolism while the CYP2C19*2 and *3 alleles are non-functional. CYP2C19*2 and *3 account for the majority of reduced function alleles in white (85%) and Asian (99%) poor metabolizers. Other alleles associated with absent or reduced metabolism are less frequent, and include, but are not limited to, CYP2C19*4, *5, *6, *7, and *8. A patient with poor metabolizer status will possess 2 loss-of-function (LOF) alleles as defined above. Published frequencies for poor CYP2C19 metabolizer genotypes are approximately 2% for whites, 4% for blacks and 14% for Chinese. Tests are available to determine a patient’s CYP2C19 genotype.

The results of this research are now included in the prescribing label for clopidogrel. The new label includes that reduced CYP2C19 metabolism in intermediate and poor metabolizers is associated with diminished response to clopidogrel and that pharmacogenetic testing can identify genotypes associated with variability in CYP2C19 activity. However, the information in the new labeling does not include a recommendation for genetic testing prior to administration of the drug and does not provide information on how to determine dosing once a patient’s individual genotype has been established.

On March 12, 2010, the labeling of Plavix was updated to include a boxed warning about the diminished effectiveness of clopidogrel in poor metabolizers. The boxed warning states that the effectiveness of Plavix is dependent on its activation to an active metabolite by the cytochrome P450 (CYP) system, principally CYP2C19. The labeling states that Plavix at recommended doses forms less of that metabolite and has a smaller effect on platelet function in patients who are CYP2C19 poor metabolizers. Citing cohort studies and retrospective analyses of clinical trials, the labeling states that poor metabolizers with acute coronary syndrome or undergoing percutaneous coronary intervention (PCI) treated with Plavix at recommended doses exhibit higher cardiovascular event rates than do patients with normal CYP2C19 function. The labeling states that tests are available to identify a patient’s CYP2C19 genotype, and that these tests can be used as an aid in determining therapeutic strategy. The labeling states that clinicians should consider alternative treatment or treatment strategies in patients identified as CYP2C19 poor metabolizers.

The new label notes that reduced CYP2C19 metabolism in intermediate and poor metabolizers is associated with diminished response to clopidogrel and that pharmacogenetic testing can identify genotypes associated with variability in CYP2C19 activity. However, the information in the new labeling does not include a recommendation for genetic testing prior to administration of the drug. The label
states, "although a higher dose regimen (600 mg loading dose followed by 150 mg once-daily) in poor metabolizers increases antiplatelet response, an appropriate dose regimen for this patient population has not been established in clinical outcome trials." In addition, the label states that there may be genetic variants of other CYP450 enzymes with effects on the ability to form clopidogrel's active metabolite.

A Clinical Alert from the American College of Cardiology and the American Heart Association (Holmes et al, 2010) stated that the clopidogrel boxed warning leaves the issue of whether to perform CYP2C19 testing up to the individual physician. It does not specifically require genetic testing or other changes in evaluation or treatment and does not imply that there are solid evidence-based reasons for such actions. Rather, it serves to make clinicians aware of the imperfect, but significant, knowledge that we have about genetic variations in response to clopidogrel and to emphasize that clinicians should use this knowledge to make decisions about how to treat individual patients. The Clinical Alert concluded that the evidence base is insufficient to recommend either routine genetic or platelet function testing at the present time. There is no information that routine testing improves outcome in large subgroups of patients. In addition, the clinical course of the majority of patients treated with clopidogrel without either genetic testing or functional testing is excellent. The Clinical Alert stated that clinical judgment is required to assess clinical risk and variability in patients considered to be at increased risk. The Clinical Alert stated that genetic testing to determine if a patient is predisposed to poor clopidogrel metabolism ("poor metabolizers") may be considered before starting clopidogrel therapy in patients believed to be at moderate or high risk for poor outcomes. This might include, among others, patients undergoing elective high-risk PCI procedures (e.g., treatment of extensive and/or very complex disease). The Clinical Alert stated that, if such testing identifies a potential poor metabolizer, other therapies, particularly prasugrel for coronary patients, should be considered. With these other therapies, the balance of potential ischemic benefit with the known increased risk of bleeding should be considered either with alternative clopidogrel dosing or newer agents such as prasugrel.

Guidelines issued in December 2009 from the American College of Cardiology, the American Heart Association, and the Society for Cardiac Angiography and Interventions stated that, although clopidogrel in combination with ASA has been shown to reduce recurrent coronary events in the post-hospitalized acute coronary syndrome (ACS) population, the response to clopidogrel varies among patients, and clopidogrel resistance has been observed (Kushner et al, 2009). The guidelines noted that information is accumulating about the variations in the anti-platelet effect of clopidogrel in patients with LOF alleles in the gene encoding CYP450 2C19. These patients form a subgroup in which failure of clopidogrel effectiveness has been linked to adverse clinical
outcomes. The guidelines stated, accordingly, that the effective clopidogrel dose for an individual undergoing PCI for STEMI may not be known. The guidelines stated that a large randomized trial is attempting to determine whether adjustment of clopidogrel therapy on the basis of platelet function testing with a point-of-care assay safely improves outcomes after PCI with drug-eluting stent (DES). The guidelines stated that the current recommended loading dose for clopidogrel is uncertain. In addition, a period of several hours is required to metabolize clopidogrel to its active metabolite, which leaves a window of time during which there is a reduced level of effectiveness even in responders.

The guidelines observed that thienopyridine prasugrel has a higher level of inhibition of platelet aggregation than clopidogrel and a more rapid onset of action (Kushner et al, 2009). The guidelines stated that its metabolism is not affected by the 2C19 allele variant. However, the guidelines do not endorse explicitly one of the thienopyridines over the other. Furthermore, there are some emerging studies that suggest there may be some patients who are resistant to clopidogrel, but there is little information about the use of strategies to select patients who might do better with prasugrel. There is not yet experience with the use of prasugrel in routine community practice. As a result, the guidelines state that there is some uncertainty regarding the net benefit and risks of one drug over another for a given patient.

More recently published evidence casts doubt over the role of genetic variation in CYP2C19 and clopidogrel treatment effects. A recent study published in the New England Journal of Medicine (Pare et al, 2010) found that, among patients with ACS or atrial fibrillation, the effect of clopidogrel as compared with placebo is consistent, irrespective of CYP2C19 genotype. Investigators genotyped patients from 2 large, randomized, placebo-controlled trials of the effect of clopidogrel on the rate of cardiovascular events among patients with ACS and among patients with atrial fibrillation. Investigators found that, in both studies, clopidogrel's superiority to placebo was similar, regardless of patients' genotype. In addition, CYP2C19 genotype was not associated with adverse events during treatment.

A randomized controlled clinical study published in the Lancet (Wallentin et al, 2010), comparing clopidogrel to ticagrelor in subjects with ACS, found no significant effect difference of clopidogrel by CYP2C19 genotype. In this study, ticagrelor was found to be more effective than clopidogrel in reducing the risk of cardiovascular events, but the effect difference did not vary significantly by CYP2C19 genotype. In addition, CYP2C19 genotype was not associated with adverse events during treatment.
Tetrabenazine (Xenazine) is a monoamine depletor for oral administration, and is indicated for the treatment of chorea associated with Huntington’s disease. The precise mechanism by which tetrabenazine exerts its anti-chorea effects is unknown, but is believed to be related to its effect as a reversible depletor of monoamines (such as dopamine, serotonin, norepinephrine, and histamine) from nerve terminals. Tetrabenazine is extensively metabolized in the liver to metabolites alpha-HTBZ and beta-HTBZ. Alpha-HTBZ and beta-HTBZ are metabolized by CYP450 enzymes, principally CYP2D6, to another major circulating metabolite, O-dealkylated-HTBZ. These metabolites are primarily renally excreted. The US Food and Drug Administration (FDA) approved Xenazine (tetrabenazine) for the treatment of chorea in people with Huntington’s disease. The FDA recommends genotyping for CYP2D6 prior to treatment for daily doses over 50mg. Dosage recommendations are indicated based on test results. The labeling of Xenazine states that, although the pharmacokinetics of tetrabenazine and its metabolites in subjects who do not express the drug metabolizing enzyme CYP2D6 (poor metabolizers) have not been systematically evaluated, it is likely that the exposure to beta-HTBZ would be increased compared to subjects who express the enzyme (extensive metabolizers), with an increase similar to that observed in patients taking strong CYP2D6 inhibitors. The labeling of Xenazine states that “[p]atients should be genotyped for CYP2D6 prior to treatment with daily doses of tetrabenazine over 50 mg”, and patients who are poor metabolizers should not be given daily doses greater than 50 mg.

Knowing how an individual will respond to warfarin would help in tailoring the dose needed to maintain appropriate anticoagulation. Toward that end, researchers studied variability in the recently discovered gene for the warfarin target, vitamin K epoxide reductase complex 1 (VKORC1). VKORC1 is the key enzyme of the vitamin K cycle and the molecular target of coumarins, which represent the most commonly prescribed drugs for therapy and prevention of thromboembolic conditions. Recent studies have identified variants of the VKORC1 gene as responsible for about 25% of the inter-individual response to warfarin, and for significant inter-ethnic response variability. Whether or not this is sufficient to successfully direct initial dosing, achieve a shorter time to stable dose, and reduce bleeding events has yet to be shown in a prospective trial. There are several such prospective clinical studies that are currently ongoing both in the United States and Europe.

Wadelius and Pirmohamed (2007) explained that the most important genes affecting the pharmacokinetic and pharmacodynamic parameters of warfarin are CYP2C9 and VKORC1. These 2 genes, together with environmental factors, partly explain the inter-individual variation in warfarin dose requirements.
Although studies have shown that genetic polymorphisms in CYP2C9 and VKORC1 affect warfarin dosing, no randomized controlled trials have linked the use of pharmacogenomic testing to improvements in clinical outcomes. An assessment by the Canadian Agency for Drugs and Technologies in Health (Ndegwa, 2007) found that most of the studies performed to date have been of retrospective or cross-sectional design. Consequently, individuals who stop warfarin early because of adverse effects or those who have difficulty attaining a therapeutic maintenance dose may have been excluded. Furthermore, many studies were underpowered to investigate the risk of bleeding.

Rieder et al (2005) analyzed genetic data from 186 American patients of European descent who were recruited from anticoagulation clinics and were receiving long-term warfarin therapy. They identified 10 single-nucleotide polymorphisms of VKORC1 that showed significant associations with warfarin maintenance doses and that had an overall frequency of more than 5% in this cohort. From these 10 single-nucleotide polymorphisms, the researchers inferred five common VKORC1 haplotypes (a haplotype is a set of closely linked genetic markers present on one chromosome that tend to be inherited together). Four of the haplotypes had independent associations with warfarin maintenance doses, 2 with a low-dose requirement and 2 with a high-dose requirement. Ultimately, subjects were linked with 1 of 3 haplotype groupings; the mean daily warfarin maintenance dose differed significantly across these 3 subgroups (2.7 mg, 4.9 mg, and 6.2 mg, respectively). The authors report that VKORC1 haplotype explained 25% of the variance in warfarin dose, and they replicated these findings in a larger European American population. The researchers also examined VKORC1 haplotype frequencies in Asian American and African American populations and found significant variability by race.

The FDA has cleared for marketing the Verigene warfarin metabolism test, manufactured by Nanosphere, which detects variants of two genes, CYP2C9 and VKORC1 that can contribute to changes in warfarin metabolism (FDA, 2007).

Large ongoing studies of genes involved in the actions of warfarin, together with prospective assessment of environmental factors, will increase the capacity to accurately predict warfarin dose. Kamali (2006) stated that prospective studies that incorporate both CYP2C9 and VKORC1 genes and environmental factors in warfarin dose calculation will be needed to demonstrate the safety, cost-effectiveness, and feasibility of individualized dosing regimens.
ECRI Institute's Health Technology Trends (2007) reported that "[a]lthough genetic testing can currently identify who has these variants [CYP2C9 and VKORC1], more studies are needed to explore the precise starting doses for these patients".

Anderson et al (2007) stated that pharmacogenetic-guided dosing of warfarin is a promising application of "personalized medicine" but has not been adequately examined in randomized studies. In a randomized trial, these investigators assessed genotype-guided versus standard warfarin dosing in patients initiating oral anticoagulation (n = 206). Buccal swab DNA was genotyped for CYP2C9 *2 and CYP2C9 *3 and VKORC1C1173T with a rapid assay. Standard dosing followed an empirical protocol, whereas pharmacogenetic-guided dosing followed a regression equation including the 3 genetic variants as well as age, sex, and weight. Prothrombin time INR was measured routinely on days 0, 3, 5, 8, 21, 60, and 90. A research pharmacist un-blinded to treatment strategy managed dose adjustments. Patients were followed-up for up to 3 months. Pharmacogenetic-guided predicted doses more accurately approximated stable doses (p < 0.001), resulting in smaller (p = 0.002) and fewer (p = 0.03) dosing changes and INRs (p = 0.06). However, percent out-of-range INRs (pharmacogenetic = 30.7 %, standard = 33.1 %), the primary end point, did not differ significantly between arms. Despite this, when restricted to wild-type patients (who required larger doses; p = 0.001) and multiple variant carriers (who required smaller doses; p < 0.001) in exploratory analyses, results (pharmacogenetic = 29 %, standard = 39 %) achieved nominal significance (p = 0.03). Multiple variant allele carriers were at increased risk of an INR of greater than or equal to 4 (p = 0.03). The authors concluded that an algorithm guided by pharmacogenetic and clinical factors improved the accuracy and efficiency of warfarin dose initiation. Despite this, the primary end point of a reduction in out-of-range INRs was not achieved. In subset analyses, pharmacogenetic guidance showed promise for wild-type and multiple variant genotypes.

A review by Hynicka et al (2007) concluded that the use of pharmacogenomic testing in the initiation of warfarin therapy does not show improved outcomes in either safety or efficacy with warfarin therapy. Studies of pharmacogenomic testing to improve outcomes with initiation of warfarin therapy were eligible for inclusion. All patients were adults; however, the included studies varied in their population and treatment regimens. The genotypes included CYP2C9 and VKORC1 wild-types and variants. Four studies met the inclusion criteria: 2 randomized controlled trials (n = 238) and 2 prospective cohort studies (n = 345). None of the studies reported a significant difference in efficacy between dosing strategies, although the prospective cohort studies reported a tendency towards fewer adverse events in patients with pharmacogenomic-guided dosing.
An assessment published by the Canadian Agency for Drugs and Technology in Health (Ndegwa, 2007) on “Pharmacogenomics and Warfarin Therapy” concluded that “prospective studies are needed to determine whether pharmacogenomic testing improves patient outcomes, identify which subgroups of patients may benefit, and clarify the risks and costs associated with the use of these tests. Several randomized controlled trials are currently evaluating the impact of pharmacogenomics on dosing accuracy, time to achieve and maintain target international normalized ratio (INR), incidence of bleeding or thromboembolic events, and monitoring requirements”.

In August 2007, the FDA updated the product label for warfarin to include information on the impact of genetic variations in CYP2C9 and VKORC1 on warfarin metabolism; however the FDA does not require the use of these genetic tests in dosing individual patients initiating warfarin therapy. On January 28, 2008, the FDA cleared the Infiniti 2C9-VKORC1 Multiplex Assay (AutoGenomic, Inc., Carlsbad, CA) for detection of Warfarin sensitivity. Guidelines for pharmacogenomics-based warfarin dosing are under development.

A technology assessment by the California Technology Assessment Forum (CTAF, 2008) reviewed the scientific evidence for the use of genetic testing to guide the initial dosing of warfarin when initiating therapy for anticoagulation. The assessment stated that genotyping studies of patients at a stable, therapeutic dose of warfarin demonstrated that patients who have CYP2C9*2 and CYP2C9*3 require lower doses of warfarin on average than patients with the more common CYP2C9*1 allele; in addition, patients with the A haplotype of VKORC1 require lower doses of warfarin on average than patients with the B haplotype. The assessment reported that these genetic variations have been shown to predict an increased risk of excessive anticoagulation and major bleeding among patients prescribed warfarin and that statistical models have been developed in an attempt to predict the dose of warfarin needed to achieve stable anticoagulation. The assessment examined the results of a small case series and 3 small, randomized clinical trials and reported that the model used by the case series did reasonably well at predicting the warfarin dose, but that patients with the CYP2C9*2 and CYP2C9*3 alleles were still at more than a 4-fold risk of excessive anticoagulation; only 1 of the randomized trials used a model incorporating genotyping information from both CYP2C9 and VKORC1 and that study found almost no difference in outcomes between patients receiving warfarin using a pharmacogenetic model and those treated according to a standard approach. The assessment stated, “Significant uncertainty remains in the field. There is no widely accepted, standard pharmacogenetic model to determine the starting dose of warfarin and new models are being developed in 2008. Current models only explain 50 % to 60 % of the variability in warfarin dosing and the remaining variability is unexplained. Only 1 of the randomized trials used a model incorporating
information from both genes with variants known to influence warfarin dosing. Furthermore, all of the trials to date have been under-powered to meaningfully evaluate the effect of genotyping on major bleeding, the most important clinical outcome." The assessment concluded that the use of genetic testing to guide initial warfarin dosing does not meet Technology Assessment Criteria 3 through 5 for safety, effectiveness and improvement in health outcomes. Several large clinical trials are ongoing in both the United States and Europe to clarify the role of genetic testing in warfarin management.

The Centers for Medicare & Medicaid Services (CMS, 2013) concluded that the available evidence does not demonstrate that pharmacogenomic testing of CYP2C9 or VKORC1 alleles to predict warfarin responsiveness improves health outcomes in Medicare beneficiaries. Therefore, CMS determined that pharmacogenomic testing of CYP2C9 or VKORC1 alleles to predict warfarin responsiveness is not reasonable and necessary.

A randomized controlled clinical trial found that genotype-guided dosing of warfarin did not improve anticoagulation control during the first four weeks of therapy. Kimmel, et al. (2013) randomly assigned 1015 patients to receive doses of warfarin during the first 5 days of therapy that were determined according to a dosing algorithm that included both clinical variables and genotype data or to one that included clinical variables only. All patients and clinicians were unaware of the dose of warfarin during the first 4 weeks of therapy. The primary outcome was the percentage of time that the international normalized ratio (INR) was in the therapeutic range from day 4 or 5 through day 28 of therapy. At 4 weeks, the mean percentage of time in the therapeutic range was 45.2% in the genotype-guided group and 45.4% in the clinically guided group (adjusted mean difference, [genotype-guided group minus clinically guided group], -0.2; 95% confidence interval, -3.4 to 3.1; P=0.91). There also was no significant between-group difference among patients with a predicted dose difference between the two algorithms of 1 mg per day or more. There was, however, a significant interaction between dosing strategy and race (P=0.003). Among black patients, the mean percentage of time in the therapeutic range was less in the genotype-guided group than in the clinically guided group. The rates of the combined outcome of any INR of 4 or more, major bleeding, or thromboembolism did not differ significantly according to dosing strategy.

**UGT1A1 Molecular Assay**

Colorectal cancer (CRC) is one of the most common malignancies in western countries showing an increasing incidence, and has been associated with genetic as well as
lifestyle factors. About 150,000 new cases of CRC are diagnosed each year in the United States, 40 to 50% of which are metastatic. Irinotecan (Camptosar) is a chemotherapeutic agent approved as a combination therapy with 5-FU/leucovorin for the treatment of advanced CRC. The response to irinotecan is variable, possibly because of individuals’ variation in the expression of the enzymes that metabolize irinotecan. Although multiple genes may play a role in irinotecan activity, the uridine diphosphate glycuronosyltransferase 1 family, polypeptide A1 (UGT1A1) enzyme has been strongly associated with irinotecan-related toxicity. The UGT1A1 gene is responsible for glucuronidation of the active metabolite of irinotecan. A common di-nucleotide repeat polymorphism in the UGT1A1 promoter region (UGT1A1*28) has been correlated with toxicity in cancer patients receiving irinotecan-containing therapy.

Lentz et al (2005) stated that hepatic metastases occur in about 50% of patients with CRC. Since hepatic metastases are often inaccessible for surgery, chemotherapy of metastases is important. The most commonly used chemotherapeutic agents for hepatic metastases are fluorouracil, irinotecan, and oxaliplatin. Several enzymes are known to be involved in the metabolism of these drugs, and the activity of these enzymes varies greatly between individuals. The causes of this variation include genetic polymorphisms, different regulation between normal and cancer tissue, and the influence of chemotherapy on enzyme expression. The varying enzyme activity may have an important effect on the outcome of chemotherapy. Lentz et al (2005) reported that several studies confirm the influence of the activity of thymidylate synthase, thymidine phosphorylase and dihydropyrimidine dehydrogenase on the outcome of fluorouracil therapy for CRC, with higher enzyme activities predicting lower treatment efficacy. Although fewer studies are available regarding therapy of hepatic metastases, the same relationship between thymidylate synthase activity and outcome of fluorouracil therapy observed for primary CRC was found. For the other 2 enzymes, only a few studies are available, but the results indicate similarly that higher enzyme activity seems to be disadvantageous. Enzymes that are responsible for the activation, metabolism, and mechanism of action of irinotecan (e.g., CYP 3A4 and UGT1A1) also exhibit variable inter-individual activity. The authors concluded therefore, that there may be an association between enzyme activity and response to therapy. For example, in patients with CRC, higher enzyme activity of topoisomerase-I seems to be predictive of a better response to irinotecan. CYP3A4 and UGT1A1 activity levels might be predictive of irinotecan toxicity rather than efficacy. These authors stated that available data indicate the importance of the different enzyme activities on the outcome of chemotherapy of hepatic metastases in CRC. The authors noted that more information is needed, especially for the newer drugs irinotecan and oxaliplatin. However, the existing data are very promising in respect to the potential to guide dose and drug
selection for more efficient and less toxic chemotherapy of hepatic metastases from CRC.

The Invader UGT1A1 molecular assay (Genzyme Corporation, Cambridge, MA) was cleared by the FDA on August 22, 2005. The test can be performed before starting irinotecan therapy and is designed to identify patients who may be at risk for ADRs to the chemotherapeutic agent by detecting a genetic variation in the UGT1A1 gene.

A clinical study (Innocenti et al, 2004) indicated that patients with one of these variations in the UGT1A1 gene have a significantly greater risk of experiencing drug-related toxicity from irinotecan than those without it. The product labeling for irinotecan (Camptosar) was updated to recommend that a reduced initial dose should be considered for patients homozygous for UGT1A1*28 allele, although the precise dose reduction in this group of patients is unknown (Waknine, 2005). The product labeling, however, does not include a recommendation for assessment of UGT1A1 status prior to initiation of irinotecan therapy.

In a prospective study, Innocenti and colleagues (2004) assessed the association between the prevalence of severe toxicity and UGT1A1 genetic variation. A total of 66 cancer patients with advanced disease refractory to other treatments received irinotecan 350 mg/m2 every 3 weeks. Toxicity and pharmacokinetic data were measured during cycle 1. UGT1A1 variants (-3279G>T, -3156G>A, promoter TA indel, 211G>A, 686C>A) were genotyped. The prevalence of grade 4 neutropenia was 9.5 %. Grade 4 neutropenia was much more common in patients with the TA indel 7/7 genotype (UGT1A1(∗)28 homozygous) (3 of 6 patients; 50 %) compared with 6/7 (3 of 24 patients; 12.5 %) and 6/6 (0 of 29 patients; 0 %) (p = 0.001). The TA indel genotype was significantly associated with the absolute neutrophil count nadir (7/7 less than 6/7 less than 6/6, p = 0.02). The relative risk of grade 4 neutropenia was 9.3 (95 %) for the 7/7 patients versus the rest of the patients. Pre-treatment total bilirubin levels were significantly higher in patients with grade 4 neutropenia (0.83 +/- 0.08 mg/dL) compared to those without grade 4 neutropenia (0.47 +/- 0.03 mg/dL; p < 0.001). The -3156G>A variant seemed to distinguish different phenotypes of total bilirubin within the TA indel genotypes. The -3156 genotype and the SN-38 area under the concentration versus time curve were significant predictors of absolute neutrophil count nadir (r² = 0.51). These investigators concluded that UGT1A1 genotype and total bilirubin levels are strongly associated with severe neutropenia, and could be used to identify cancer patients predisposed to the severe toxicity of irinotecan. Furthermore, the hypothesis that the -3156G>A variant is a better predictor of UGT1A1 status than the previously reported TA indel requires further testing.
In an accompanying editorial, McLeod and Watters (2004) raised questions regarding the findings of Innocenti et al (2004):

I. is the relative risk of grade 4 neutropenia in a patient with the UGT1A1(*28) homozygous genotype the same after 300 to 350 mg/m2 every 3 weeks (9.3-fold risk) as after the 100 to 125 mg/m2 weekly regimen? and

II. does this marker retain its predictive power when irinotecan is used as part of a combined treatment?

These researchers also noted that it would be difficult to perform randomized controlled trials to ascertain if there is a predictable, safe, and effective dose of irinotecan that can be given to patients with the UGT1A1(*28) homozygous genotype, or if physicians should choose a non-irinotecan-containing regimen, since only 10% of all patients have this genotype. Moreover, studies are planned to determine the impact of dosage on the safety of irinotecan in patients with either the UGT1A1 6/6/ or 6/7 genotype, which are the 2 most common genotypes in the general patient population.

While several investigators (And et al, 2000; Iyer et al, 2002, and Innocenti et al, 2004) reported that testing of patients carrying the UGT1A1*28 polymorphism may detect their susceptibility to irinotecan-related toxicity, others (Marcuello et al, 2004, Carlini et al, 2005, Dervieux et al, 2005, and Eichelbaum et al, 2005) have questioned its clinical value. In a clinical trial, Marcuello et al (2004) examined the influence of the UGT1A1 gene promoter polymorphism in the toxicity profile, in the response rate and in the overall survival (OS) in 95 patients with metastatic CRC treated with an irinotecan-containing chemotherapy. Genotypes were determined by analyzing the sequence of TATA box of UGT1A1 of genomic DNA from the patients. Clinical parameters and genotypes were compared by uni-variate and multi-variate statistical methods. The more frequent ADRs were asthenia (n = 34), diarrhea (n = 29) and neutropenia (n = 20). Severe diarrhea was observed in 7/10 (70%) homozygous and 15/45 (33%) heterozygous in comparison to 7/40 (17%) wild-type patients (p = 0.005). These results
maintained the statistical significance in logistic regression analysis (p = 0.01) after adjustment for other clinical relevant variables. The presence of severe hematological toxicity increased from wild-type patients to UGT1A1(*)28 homozygotes, but without achieving statistical significance. No relationship was found between the UGT1A1(*)28 genotypes and infection, nausea or mucositis. In uni-variate studies, patients with the UGT1A1(*)28 polymorphism showed a trend to a poorer OS (p = 0.09). In the multi-variate analysis, the genotype was not related to clinical response or to OS. These investigators stated that the role of the UGT1A1 genotype as a predictor of toxicity in patients with CRC receiving irinotecan demands the performance of randomized controlled studies to determine if genotype-adjusted dosages of the drug can help to establish safe and effective doses not only for patients with the UGT1A1(*)28 homozygous genotype, but also for those with the most common UGT1A1 6/6 or 6/7 genotype.

In a phase II clinical study, Carlini and co-workers (2005) examined whether germ-line polymorphisms within genes related to drug target (thymidylate synthase) or metabolizing enzymes (UGT isozymes) would alter response and toxicity to the combination of capecitabine plus irinotecan. A total of 67 patients with measurable CRC were treated with intravenous irinotecan (100 or 125 mg/m2) on days 1 and 8 and capecitabine orally (900 or 1,000 mg/m2, twice daily) on days 2 through 15 of each 3-week cycle. Genomic DNA was obtained from peripheral blood and genotyped using Pyrosequencing, GeneScan, and direct sequencing technologies. The overall objective response rate was 45 % with 21 patients (31 %) exhibiting grade 3 or 4 diarrhea and 3 patients (4.5 %) demonstrating grade 3 or 4 neutropenia in the first 2 cycles. Low enzyme activity UGT1A7 genotypes, UGT1A7*2/*2 (6 patients) and UGT1A7*3/*3 (7 patients), were significantly associated with anti-tumor response (p = 0.013) and lack of severe gastrointestinal toxicity (p = 0.003). In addition, the UGT1A9 -118 (dT)(9/9) genotype was significantly associated with reduced toxicity (p = 0.002) and increased response (p = 0.047). There were no statistically significant associations between UGT1A1, UGT1A6, or thymidylate synthase genotypes and toxicity or tumor response. The authors concluded that these data strongly suggest that UGT1A7 and/or UGT1A9 genotypes may be predictors of response and toxicity in CRC patients treated with capecitabine plus irinotecan. Specifically, patients with genotypes conferring low UGT1A7 activity and/or the UGT1A9 (dT)(9/9) genotype may be particularly likely to exhibit greater anti-tumor response with little toxicity. However, it is interesting to note that the allele frequencies of UGT1A7 gene in Taiwan Chinese are different from those in Caucasians and Japanese (Huang et al, 2005).

In a review, Dervieux et al (2005) stated that several proofs of principle have established that pharmacogenetic testing for mutations altering expression and functions of genes
associated with drug disposition and response can reduce the "trial-and-error" dosing and decrease the risk of ADRs. These proofs of principle include UGT1A1 and irinotecan therapy, as well as CYP450 2C9 and S-warfarin therapy. These evidences advocate for the prospective identification of mutations associated with drug response, serious ADRs and treatment failure. The authors stated that with the convergence of rising drug costs and evidence supporting the clinical benefits of pharmacogenetic testing, it will be important to demonstrate the improved net health outcomes attributed to the additional costs for this testing.

This in agreement with the observation of Eichelbaum et al (2005) who noted that there is also a growing list of genetic polymorphisms in drug targets that have been demonstrated to influence drug response. A major limitation that has moderated the use of pharmacogenetic testing in the clinical setting is the lack of prospective clinical trials showing that such testing can improve the benefit/risk ratio of drug therapy. Moreover, Gardiner and Begg (2005) stated that currently pharmacogenetic tests for drug metabolizing enzymes are rarely carried out in clinical practice, despite repeated claims that they may benefit patient care. They noted that "the only tests performed with any regularity in Australasia are for thiopurine methyltransferase and pseudocholinesterase, and CYP2D6 phenotyping in 1 center for patients on perhexilene. The low clinical utilization reflects a poor evidence base, un-established clinical relevance and, in the few cases with the strongest rationale, a slow translation to the clinical setting".

Han et al (2006) determined if uridine diphosphate-glucuronosyltransferase 1A1, UGT1A7, and UGT1A9 polymorphisms affect the pharmacokinetics (PK) of irinotecan and treatment outcome of Korean patients with advanced non-small-cell lung cancer (NSCLC). A total of 81 patients with advanced NSCLC were treated with irinotecan (80 mg/m²) on day 1 and 8 and cisplatin (60 mg/m²) on day 1 intravenously of each 3-week cycle. Genomic DNA was extracted from peripheral blood and genotyped using direct sequencing. These researchers analyzed the association of UGT1A genotypes with irinotecan PK and clinical outcomes. All statistical tests were two-sided. In genotype-PK association analysis, UGT1A1*6/*6 (n = 6), UGT1A7*3/*3 (n = 6), and UGT1A9-118(dT)9/9 (n = 11) were associated with significantly lower area under the time-concentration curve (AUC) SN-38G to SN-38 (AUC(SN-38G)/AUC(SN-38)) ratio (p = 0.002, p = 0.009, and p = 0.001, respectively). In linkage disequilibrium analysis, the UGT1A7 variants were highly linked with the UGT1A1*6 (D' = 0.85, r² = 0.63) and UGT1A9*22 (D' = 0.95, r² = 0.88), which was substantiated in haplotype analysis. Patients with UGT1A1*6/*6 had lower tumor response and higher incidence of severe neutropenia. UGT1A9-118(dT)9/9 also showed a trend for high incidence of severe diarrhea, but not tumor response. In survival analysis, patients with UGT1A1*6/*6 had
significantly shorter progression-free survival ($p = 0.001$) and overall survival ($p = 0.017$). The authors concluded that UGT1A1*6 and UGT1A9*22 genotypes may be important for SN-38 glucuronidation and associate with irinotecan-related severe toxicity. Specifically, UGT1A1*6 might be useful for predicting tumor response and survival outcome of Korean patients with NSCLC treated with irinotecan-based chemotherapy. These investigators also stated that "[a]lthough it is still hypothetical, we suggest that UGT1A1*6 and/or UGT1A9*22 genotypes might be important for predicting severe toxicity and treatment outcome after irinotecan-based chemotherapy. To confirm the data observed in this study, further larger studies are needed in an independent data set, preferably in a group of patients of similar ethnicity".

In an editorial that accompanied the study by Han et al (2006), Innocenti and associates (2006) stated that "[t]he study by Han et al provides evidence that the UGT1A1*6 polymorphism can be considered a biomarker of severe toxicity of irinotecan in Asians. The impact of this variant on efficacy in irinotecan-containing regimens should be prospectively investigated in patients of Asian descent ....".

In a review on genetic polymorphisms of drug-metabolizing enzymes and drug transporters in the chemotherapeutic treatment of cancer, Bosch et al (2006) focused on the clinical significance of polymorphisms in drug-metabolizing enzymes (CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP3A5, dihydropyrimidine dehydrogenase, UGT1A1, glutathione S-transferase, sulfotransferase [SULT] 1A1, N-acetyltransferase [NAT], thiopurine methyltransferase [TPMT]) and drug transporters (P-glycoprotein [multi-drug resistance 1], multi-drug resistance protein 2 [MRP2], breast cancer resistance protein [BCRP]) in influencing toxicity and effectiveness of chemotherapy. The authors stated that the clinical application of pharmacogenetics in cancer treatment will require more detailed information of the different polymorphisms in drug-metabolizing enzymes and drug transporters; and that larger studies, in different ethnic populations, and extended with haplotype and linkage disequilibrium analysis, are needed for each anti-cancer drug separately.

While initial reports described above suggested that UGT1A1*28 homozygotes were at high risk for worse irinotecan-related hematologic and gastrointestinal toxicity, more recent reports suggest that the magnitude of the problem (particularly the association with worse diarrhea) is not as great as was initially suspected. In a prospective study of 250 patients with metastatic colorectal cancer starting irinotecan, fluorouracil and leukovorin, the relative risk for grade 3 or 4 hematologic toxicity was significantly higher among UGT1A1*28 homozygotes (odds ratio 8.63, 95 % CI: 1.31 to 56.55) (Toffoli et al, 2006). However, the absolute magnitude of risk was relatively low (13.6 % versus 1.7 %
for those with the wild-type alleles), and relevant for the first cycle only. Furthermore, there was no significant association between the presence of a UGT1A1*28 polymorphism and severity of diarrhea, or the need for irinotecan dose reduction.

One study found higher rates of neutropenia in persons homozygous for the UGT1A1*28 allele, regardless of whether the combination chemotherapy regimen included irinotecan (McLeod et al, 2006). In a preliminary analysis of data from 520 patients with colorectal cancer enrolled in the United States Intergroup (INT) 9741 trial, which compared a variety of first-line oxaliplatin and irinotecan-containing chemotherapy regimens, the risk of grade 3 or 4 neutropenia was significantly higher for homozygotes (but not heterozygotes) regardless of whether they received irinotecan or oxaliplatin-based chemotherapy (36.2 % versus 18.2 % and 14.8 % for homozygotes, heterozygotes, and wild-type alleles, respectively). Similar to the study by Toffoli et al (2006) described above, the investigators found no association between inheritance of UGT1A1*28 alleles and treatment-related diarrhea. UGT1A1*28 was also not a predictor of tumor response, time to progression, or overall survival.

The Evaluation of Genomic Applications in Practice and Prevention Working Group (EGAPP, 2009) found that the evidence is currently insufficient to recommend for or against the routine use of UGT1A1 genotyping in patients with metastatic colorectal cancer who are to be treated with irinotecan, with the intent of modifying the dose as a way to avoid adverse drug reactions (severe neutropenia). The EGAPP Working Group found no evidence to support clinical utility. Preliminary modeling suggests that, even if targeted dosing were to be highly effective, it is not clear that benefits (reduced adverse drug events) outweigh harms (unresponsive tumors).

In summary, although it has been advocated that pharmacogenetic testing of patients with CRC before chemotherapy with irinotecan may reduce the frequency of severe toxicities by allowing alternate therapy selections for patients carrying the UGT1A1*28 polymorphism, the clinical value of this testing (i.e., whether testing will lead to better health outcomes) has yet to be established by prospective, randomized, controlled trials. Only about 1 in 10 patients will be identified as being homozygous, and the excess risk of severe neutropenia that is attributable to the inheritance of this polymorphism appears to be small. There is a lack of consensus on whether initial dose reduction is needed for UGT1A1*28 homozygotes, and the precise dose reduction that is warranted in this patient population has not been determined.

**Genotyping for HLA-B*5801**
Human leukocyte antigen-B (HLA-B) gene variations are associated with adverse reactions to some medications. Before taking allopurinol, HLA-B*5801 testing may be done for individuals of Korean descent with stage 3 or worse chronic kidney disease or of Han-Chinese or Thai descent.

**Genotyping for HLA-B*1502**

Carbamazepine (brand names Carbatrol, Equetro and Tegretol) is used for the treatment of patients with epilepsy, bipolar disorder, and neuropathic pain. The use of carbamazepine is associated with rare but severe and sometimes life-threatening skin reactions, which includes toxic epidermal necrolysis and Stevens-Johnson syndrome, characterized by multiple skin lesions, blisters, fever, itching and other symptoms. The risk of these reactions is estimated to be about 1 to 6 per 10,000 new users of the drug in countries with mainly white populations. However, the risk is estimated to be about 10 times higher in some Asian countries. Studies have demonstrated a strong association between certain serious skin reactions and an inherited variant of an immune system gene, HLA-B*1502, found almost exclusively among individuals with Asian ancestry (Hung et al, 2006; Chung et al, 2007).

Before taking carbamazepine, HLA-B*1502 testing may be used in individuals of Asian ancestry to identify an increased risk of developing severe skin disorders (eg, Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis [TEN]). Additionally, individuals who have tested positive for HLA-B 1502 may be directed to a medication other than phenytoin.

In December 2007, the FDA announced that manufacturers of drugs containing carbamazepine have agreed to add to the drugs' labeling a recommendation that, before starting therapy with the drugs patients with Asian ancestry should receive genotyping of HLA-B*1502.

**HLA-B*5701 Screening**

HLA-B*5701 screening is indicated prior to initiation of an abacavir-containing regimen to reduce the risk of a hypersensitivity reaction in HIV individuals.

Abacavir (Ziagen) is a nucleoside analogue reverse transcriptase inhibitor indicated for use in combination with other antiretroviral drugs for the treatment of HIV-1 infection. Review of reports of hypersensitivity in patients receiving abacavir (Glaxo Wellcome Inc., Research Triangle Park, NC) indicated that respiratory symptoms
(including cough, dyspnea, and pharyngitis) have occurred in approximately 20% of patients who have had hypersensitivity reactions. The frequency of the HLA-B*5701 allele varies in different populations, occurring in whites 5 to 8%, Hispanics 4 to 7%, Asians less than 1%, Spaniards 1 to 4%, and rarely in Sub-Saharan Africans. A delay in diagnosis of hypersensitivity can result in abacavir being continued or re-introduced, leading to more severe hypersensitivity reactions, including life-threatening hypotension and death.

In a double-blind, prospective, randomized study, Mallal et al (2008) examined whether HLA-B*5701 screening could prevent hypersensitivity reaction to abacavir. Patients who were infected with HIV-1 infection (n = 1956) who had not previously received abacavir were randomly assigned to undergo prospective HLA-B*5701 screening, with exclusion of HLA-B*5701 positive patients from abacavir treatment (prospective screening group), or to undergo a standard of care approach of abacavir use without prospective HLA-B*5701 screening (control group). All patients who started abacavir were observed for 6 weeks. Epicutaneous patch testing with abacavir was performed to immunologically confirm and enhance the specificity of the clinical diagnosis of hypersensitivity reaction to abacavir. The prevalence of HLA-B*5701 in this predominantly white study population was 5.6%. Hypersensitivity reaction was diagnosed in 93 patients with a significantly lower incidence in the prospective-screening group (3.4%) than in the control group (7.8%). Of the patients receiving abacavir, 72% were men, 84% were white, and 18% had not previously received anti-retroviral therapy. Screening eliminated immunologically confirmed hypersensitivity reaction (0% in the prospective-screening group versus 2.7% in the control group), with a negative predictive value of 100% and a positive predictive value of 47.9%. The authors concluded that HLA-B*5701 screening reduced the risk of hypersensitivity reaction to abacavir. Although the population in Mallal's study was predominantly white, other investigators have reported comparable sensitivity results of HLA-B*5701 for abacavir hypersensitivity in different ethnic groups, including blacks (Saag M et al, 2007) and Spaniards (Rodríguez-Nóvoa et al, 2007). In addition, Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents (2008) developed by the Department of Health and Human Services recommends:

- Screening for HLA-B*5701 before starting patients on an abacavir-containing regimen to reduce the risk of hypersensitivity reaction (strength of recommendation: Strong evidence with at least 1 randomized trial with clinical results);
- HLA-B*5701-positive patients should not be prescribed abacavir (strength of recommendation: Strong evidence with at least 1 randomized trial with clinical results);
- The positive status should be recorded as an abacavir allergy in the patient’s medical record (strength of recommendation: Strong evidence with clinical trials with laboratory results);
- When HLA-B*5701 screening is not readily available, it remains reasonable to initiate abacavir with appropriate clinical counseling and monitoring for any signs of hypersensitivity reaction (strength of recommendation: Optional with expert opinion).

**Genotyping for Apolipoprotein E (Apo E)**

Apolipoprotein E (Apo E), a member of the apolipoprotein gene family, is essential in the formation of very low density lipoprotein (VLDL) and chylomicrons. Among the variants of this gene, alleles e2, e3, and e4 are the common polymorphism found in most populations. Of these variants, Apo e3 is the most frequent (greater than 60 %) in all populations studied. The polymorphism has functional effects on lipoprotein metabolism mediated through the hepatic binding, uptake, and catabolism of chylomicrons, chylomicron remnants, VLDL, and high density lipoprotein subspecies. Apolipoprotein E is the primary ligand for 2 receptors, the low density lipoprotein (LDL) receptor (also known as the B/E receptor) found on the liver and other tissues and an Apo E-specific receptor found in the liver. The interactions of these lipoprotein complexes with their receptors form the basis for the metabolic regulation of cholesterol. Allelic variation in apolipoprotein is consistently associated with plasma concentrations of total cholesterol, LDL cholesterol, and Apo B (the major protein of LDL, VLDL, and chylomicrons). Apolipoprotein has been studied in disorders associated with elevated cholesterol levels or lipid derangements (i.e., hyperlipoproteinemia type III, coronary heart disease, strokes, peripheral artery disease, and diabetes mellitus). The apolipoprotein genotype yields poor predictive values when screening for clinically defined atherosclerosis despite positive, but modest associations with plaque and coronary heart disease outcomes. In addition to genotype-phenotype associations with vascular disease, the alleles and isoforms of apolipoprotein have been related to dementias, most commonly Alzheimer’s disease.

Several studies have been published assessing the interactions between Apo E and cholesterol in response to lipid-lowering drugs.

Gerdes et al (2000) examined whether the beneficial effects of simvastatin treatment differed by apolipoprotein genotype. After providing dietary advice, they randomized men and women aged 35 to 70 years with a history of myocardial infarction or angina, serum total cholesterol concentrations in the range of 5.5 to 8.0 mmol/L, and serum triglyceride levels of less than 2.5 mmol/L to placebo or simvastatin groups. Simvastatin
treatment reduced the mortality risk more in e4 carriers than in other patients, although the difference was not statistically significant for the treatment by genotype interaction.

At least 2 other studies have examined the influence of the Apo E polymorphism in response to lipid-lowering drug treatments in patients with combined hyperlipoproteinemia and familial hypercholesterolemia.

Knijff et al (1990) examined the influence of the Apo E polymorphism on pre-treatment plasma lipid levels and on the response to simvastatin treatment in a sample of 120 Dutch patients with heterozygous familial hypercholesterolemia. They found that differences in pre-treatment lipid levels were not related to the Apo E polymorphism in these patients. With respect to the effect of 12 weeks of simvastatin treatment, a reduction of 33 %, 38 %, and 19 % (on average) was found in the plasma levels of total cholesterol, LDL cholesterol, and triglycerides, respectively. Inter-individual variation in response to simvastatin treatment was not related to the Apo E polymorphism.

Nestel et al (1997) conducted a cross-over, randomized trial to examine the efficacy of simvastatin and gemfibrozil in patients with combined hyperlipoproteinemia. Efficacy was noted after 6 and 12 weeks on each treatment for the 66 subjects enrolled. The lipid-lowering responsiveness was greatest in those with the Apo E2 isoform with both medications.

An Agency for Healthcare Research and Quality (AHRQ, 2008) technology assessment on pharmacogenetic testing reviewed the available evidence of Apo E genotype (e2, e3, and e4) and statin treatment and found that genotyping for Apo E has not been shown to help select patients for treatment. "No studies addressed the effects of therapeutic choice: there were no data on the benefits, harms, or adverse effects on patients from subsequent therapeutic management after pharmacogenetic testing for the three Apo E genotypes."

The AHRQ assessment found that the pooled reduction in total and LDL cholesterol from baseline values was lower for all 3 genotypes but did not differ significantly among them.

The AHRQ also found significant between-study heterogeneity. "Although few studies included certain subgroups, factors that may affect the associations between all three Apo E genotypes and response to statin therapy were ethnicity, sex, familial hyperlipidemia, the type of statin used, and possibly the presence of diabetes."
In addition, there are no prospective data showing improved clinical management of hypercholesterolemia patients as a result of genotyping for Apo E.

**Genotyping for MethyleneTetrahydrofolate Reductase (MTHFR)**

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme regulating intracellular folate levels, which in turn affects DNA synthesis and methylation. Two MTHFR gene polymorphisms, C677T and A1298C, influence the metabolism of folates and could modify the pharmacodynamics of antifolates and many other drugs whose metabolism, biochemical effects, or target structures require methylation reactions. Several studies have shown these two polymorphisms may reduce cancer susceptibility and increase drug-related toxicity when folate antagonists (e.g., methotrexate, fluorouracil) are utilized, but data are inconsistent and contradictory. According to the National Cancer Institute, 5 of 6 patients who experienced grade-4 toxicity in their first cycle of adjuvant chemotherapy with cyclophosphamide, methotrexate and 5-FU for early breast cancer had the variant C677T MTHFR genotype.

An Agency for Healthcare Research and Quality (AHRQ, 2008) technology assessment on pharmacogenetic testing reviewed the available evidence on MTHFR gene polymorphisms for their associations with patient's response to therapy with antifolate chemotherapy and found that the evidence indicates that MTHFR gene polymorphisms do not predict response to chemotherapy.

AHRQ found limited data on MTHFR gene testing and therapeutic choice which preclude making meaningful inferences about the relationship between common variants in MTHFR and chemotherapy of the folate metabolic pathway.

AHRQ also found considerable variation in study designs, study populations, medication dosages, and the type of medications.

Studies have shown that MTHFR polymorphisms may affect the sensitivity to antifolate chemotherapy, however, there is insufficient evidence of its clinical effectiveness.

**Measurement of Thromboxane Metabolites in Urine**

The U.S. Preventive Services Task Force (USPSTF, 2009) has developed guidelines for chronic aspirin therapy based upon a person's age and Framingham risk score. Long-term aspirin administration has clear benefits for the secondary prevention of cardiovascular diseases with a significant 21 % reduction in the risk of cardiovascular
events over 2 years (Berger et al, 2008). However, this indicates that not all individuals respond equally to aspirin therapy and cardiovascular events may occur during aspirin therapy. This is often described as "clinical aspirin resistance". A systematic review and meta-analysis on aspirin resistance indicated that patients who are resistant to aspirin are at a greater risk (odds ratio [OR]: 3.85) of clinically important cardiovascular morbidity than patients who are sensitive to aspirin (Krasopoulos et al, 2008). The effect of aspirin administration varies considerably among patients at high risk for cardiovascular events. Gum and co-workers (2001) found insufficient inhibition of platelet aggregation by aspirin in 6 to 24 % of patients with stable coronary artery disease, while other estimates range from 5 to 60 % (Martin and Talbert, 2005).

Many authors believe that aspirin resistance can be detected by biochemical tests and several commercially available products are being marketed for this purpose. Tests used in research laboratories are aggregometry (turbidometric and impedance), tests based on activation-dependent changes in platelet surface, and tests based on activation-dependent release from platelets. Point-of-care tests include PFA-100, IMPACT, and VerifyNow, which can detect platelet dysfunction that may be due to aspirin effect.

It has been proposed that aspirin resistance can also be detected by thromboxane metabolites in urine. Aspirin inhibits platelet activation through the permanent inactivation of the cyclooxygenase (COX) activity of prostaglandin H synthase-1 (referred to as COX-1), and consequently inhibits the biosynthesis of thromboxane A₂ (TXA₂), a platelet agonist. The urinary concentrations of the metabolite 11-dehydrothromboxane B₂ (11 dhTxB₂) indicate the level of TXA₂ generation.

Eikelboom et al (2002) studied whether aspirin resistance, defined as failure of suppression of thromboxane generation, increases the risk of cardiovascular events in a high-risk population. Baseline urine samples were obtained from 5,529 Canadian patients enrolled in the Heart Outcomes Prevention Evaluation (HOPE) Study. Using a nested case-control design, the investigators measured urinary 11 dhTxB₂ levels, a marker of in vivo thromboxane generation, in 488 cases treated with aspirin who had myocardial infarction, stroke, or cardiovascular death during 5 years of follow-up and in 488 sex- and age-matched control subjects also receiving aspirin who did not have an event. After adjustment for baseline differences, the odds for the composite outcome of myocardial infarction, stroke, or cardiovascular death increased with each increasing quartile of 11 dhTxB₂, with patients in the upper quartile having a 1.8-times-higher risk than those in the lower quartile (OR, 1.8; 95 % CI: 1.2 to 2.7; p = 0.009). Those in the upper quartile had a 2-times-higher risk of myocardial infarction (OR, 2.0; 95 % CI: 1.2 to 3.4; p = 0.006) and a 3.5-times-higher risk of cardiovascular death (OR, 3.5; 95 % CI: 1.7 to 7.4; p < 0.001) than those in the lower quartile. The authors concluded that in
aspirin-treated patients, urinary concentrations of 11 dhTxB₂ predict the future risk of myocardial infarction or cardiovascular death and that these findings raise the possibility that elevated urinary 11 dhTxB₂ levels identify patients who are relatively resistant to aspirin and who may benefit from additional anti-platelet therapies or treatments that more effectively block in vivo thromboxane production or activity. However, Altman et al (2004) reviewed this study and stated that the authors support the view that failure to suppress thromboxane generation defines aspirin resistance and that this hypothesis assumes a direct association between the rise of urinary 11 dhTxB₂ levels and increment of vascular events (e.g., myocardial infarction, stroke and cardiovascular death). Altman and colleagues explained that failure of aspirin to produce the expected inhibition of platelet function might be attributed to several mechanisms and that it can not be defined by the level of serum thromboxane or its urinary metabolites because these measurements do not correlate with the reduction of inhibition of platelet aggregation in response to multiple stimuli, and also because

1. although most of the thromboxane is believed to come from the platelets, there are additional cellular origins (e.g., monocytes/macrophages are also a rich source of TXA₂),

II. unlike the platelet, the macrophage is capable of synthesizing new COX-2 after aspirin has inhibited it; COX-2 is the enzyme responsible for most of the metabolism of arachidonic acid in the macrophage, and low dose aspirin is not sufficient to inhibit COX-2 maximally,

II. macrophages in atheromata may contribute significantly to the pool of TXA₂, and

IV. aspirin only inhibits monocyte PGHS-2, which is inducible by inflammatory stimuli, transiently at very high concentrations.

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The AspirinWorks Test Kit (Corgenix Medical Corp; Broomfield, CO) is an enzyme-linked immunoassay test that can be used to determine levels of 11 dhTxB₂ in human urine. AspirinWorks received 510(k) marketing clearance from the FDA in May, 2007 and is intended to aid in the qualitative detection of aspirin in apparently healthy individuals post ingestion.

The AspirinWorks Test Kit was compared to the Accurnetrics VerifyNow Aspirin Assay as the predicate device. The manual AspirinWorks Test Kit measures urinary 11 dhTxB₂, while the automated Accurnetrics VerifyNow Aspirin Assay is a turbidimetric-based optical detection system, which measures platelet-induced aggregation in whole blood. The two devices have similar intended uses in that they both measure aspirin effect. The AspirinWorks kit detects a metabolite of TxA₂, a direct inducer of platelet aggregation, while the Accurnetrics kit measures ex vivo platelet aggregation caused by TxA₂ by artificially inducing aggregation and measuring an optical signal. Ultimately, both are analyzing aspirin’s effect through the reduction of TxA₂ production or the resulting inhibition of platelet aggregation.

According to the FDA, 2 different clinical studies were employed for the evaluation of the AspirinWorks Test Kit. Results from these studies established a cutoff for aspirin effect at less than 1500 pg 11d hTxB₂/mg creatinine. Further analysis revealed that 180/204 (88.2 %) of samples from individuals not taking aspirin were above the cut-off value. Analysis of samples from individuals taking various doses of aspirin revealed that 7/163 (4.3 %) of 81 mg/day aspirin users indicated a lack of aspirin effect (greater than 1500 pg 1 ldhTxB₂/mg creatinine) and 4/38 (10.5 %) of the 325 mg/day aspirin users indicated a lack of aspirin effect. In total, 11/201 (5.5 %) of all aspirin users tested indicated a lack of aspirin effect. These percentages are consistent with those in published literature for aspirin non-responsiveness or lack of aspirin effect.

Lordkipanidze et al (2007) compared the results obtained from 6 major platelet function tests in the assessment of the prevalence of aspirin resistance in patients with stable coronary artery disease. Patients with stable coronary artery disease (n = 201) receiving daily aspirin therapy (80 mg or more) were recruited. Platelet aggregation was measured by:
I. light transmission aggregometry (LTA) after stimulation with 1.6 mM of arachidonic acid (AA),

II. LTA after adenosine diphosphate (ADP) (5, 10, and 20 microM) stimulation,

III. whole blood aggregometry,

IV. PFA-100, and

V. VerifyNow Aspirin; urinary 11 dhTxB₂ concentrations were also measured.

A total of 8 patients (4 %, 95 % CI: 0.01 to 0.07) were deemed resistant to aspirin by LTA and AA. The prevalence of aspirin resistance varied according to the assay used: 10.3 to 51.7 % for LTA using ADP as the agonist, 18.0 % for whole blood aggregometry, 59.5 % for PFA-100, 6.7 % for VerifyNow Aspirin, and finally, 22.9 % by measuring urinary 11 dhTxB₂ concentrations. Results from these tests showed poor correlation and agreement between themselves. The authors concluded that platelet function tests are not equally effective in measuring aspirin's anti-platelet effect and correlate poorly amongst themselves and that the clinical usefulness of the different assays to classify correctly patients as aspirin resistant remains undetermined.

Hedegaard et al (2009) assessed the use of optical platelet aggregation versus thromboxane metabolites in healthy individuals and patients with stable coronary artery disease after low-dose aspirin administration. The authors investigated whether 75 mg
of daily non-enteric coated aspirin would completely inhibit the platelet cyclooxygenase-1 activity to a comparable extent in healthy individuals and stable coronary artery disease (CAD) patients. Serum thromboxane B₂ (S-TxB₂), urinary 11 dhTxB₂ (U-TxM) and arachidonic acid-induced optical platelet aggregometry (OPA) were compared in 44 coronary artery disease (CAD) patients on aspirin and in 22 healthy individuals before and after aspirin. Optical platelet aggregometry was performed in duplicate for 4 consecutive days during aspirin treatment after 1 week of treatment. Compliance was optimized by face-to-face interviews and pill counting and confirmed by S-TxB₂ measurements. The authors found that aspirin inhibited S-TxB₂ in healthy individuals (greater than 99 %; median 1.1 ng/mL, inter-quartile range [IQR] = 0.8;1.9 after aspirin) and in patients, S-TxB₂ was reduced to a similar level (0.9 ng/mL (0.7;1.5)). Healthy individuals had a median U-TxM of 278.5 pg/mg creatinine (229.5;380.0) before aspirin and 68.5 pg/mg creatinine (59.0;99.7) on aspirin corresponding to an average 74 % inhibition of the endogenous TxA₂ biosynthesis. In patients median U-TxM was 67.5 pg/mg creatinine (54.0;85.5). Seven study participants (11 %) were aspirin low-responders according to OPA, but none had S-TxB₂ in the highest quartile. The authors concluded that low-dose aspirin suppressed S-TxB₂ to comparable levels in CAD patients and healthy individuals. The authors found that despite an almost complete inhibition of S-TxB₂, some participants were low-responders according to OPA. The authors concluded that thorough compliance control and use of thromboxane-specific assays are important when measuring platelet response to aspirin.

While some investigators believe that aspirin resistance can be detected by thromboxane metabolites in urine, other investigators support the view that aspirin resistance can not be defined by the level of serum thromboxane or its urinary metabolites because these measurements do not correlate with the reduction of inhibition of platelet aggregation in response to multiple stimuli as well as various other factors. Investigators have found a number of variables that may impact an individual's response to aspirin, including patient's compliance, dose, smoking, hyperlipidemia, hyperglycemia, acute coronary syndrome, percutaneous revascularization, recent stroke, extracorporeal circulation, heart failure, exercise, circadian rhythm, absorption, concomitant medications, and polymorphisms.

Many issues are yet to be resolved in order to apply the concept of "aspirin resistance" to actual clinical practice. The clinical usefulness of a test that measures thromboxane metabolites in urine has yet to be determined. The relevance of the various ex vivo functional indexes of platelet capacity to in vivo platelet activation and the precise mechanisms underlying aspirin resistance are still largely unknown. Further investigation is needed regarding strategies to identify and treat patients resistant to aspirin.
Genetic Testing for rs3798220 Allele (LPA-Aspirin Check)

LP(a) aspirin genotype testing (eg, Cardio IQ LPA Aspirin Genotype Test and LPA-Aspirin Genotype Test) has been proposed to identify individuals at risk of cardiovascular disease (CVD) which may respond to aspirin therapy.

Investigators have described a non-synonymous single nucleotide polymorphism (SNP rs3798220) in apo(a) gene (LPA) that has been associated with increased plasma levels of Lp(a) and advanced coronary artery disease. Observational studies have also associated that this polymorphism may identify a subgroup of patients who benefit from chronic aspirin therapy in regard to a reduction in clinical coronary heart disease events, and a subgroup which reveals no benefit and thus an increased risk for gastrointestinal bleeding. A genetic testing for the rs3798220 allele (LPA-Aspirin Check, Celera, Alameda, CA) is commercially available. This SNP, encoding an isoleucine to methionine substitution in the protease-like domain of apo(a) at amino acid 4399 (I4399M), has been associated with both elevated levels of Lp(a) and cardiovascular disease. In the Women's Health Study, carriers of this apolipoprotein(a) variant had elevated Lp(a), doubled cardiovascular risk, and appeared to benefit more from aspirin than non-carriers (Chasman et al, 2009). Chasman and colleagues (2009) investigated whether this allele was associated with elevated Lp(a) and cardiovascular risk in a post hoc analysis of the Women's Health Study, a randomized trial of low-dose aspirin, and whether aspirin reduced cardiovascular risk in minor allele carriers. The investigators determined genotypes of rs3798220 for 25,131 initially healthy Caucasian participants. Among women with successful genotyping, the minor allele of rs3798220 in the LPA gene was carried by 906 (3.6 %) heterozygotes and 15 (0.06 %) homozygotes for a minor allele frequency of 1.9 %. Median Lp(a) levels at baseline were 10.0, 79.5, and 153.9mg/dL for major allele homozygotes, heterozygotes, and minor allele homozygotes, respectively (p < 0.0001). During the 9.9 years of follow-up, minor allele carriers (3.7 %) in the placebo group had 2-fold higher risk of major cardiovascular events than non-carriers (age-adjusted hazard ratio (HR) = 2.21, 95 % CI: 1.39 to 3.52). The investigators found that, among carriers, risk was reduced more than 2-fold by aspirin: for aspirin compared with placebo the age-adjusted HR was 0.44 (95 % CI: 0.20 to 0.94); risk was not significantly reduced among non-carriers (age-adjusted HR = 0.91, 95 % CI: 0.77 to 1.08). The investigators reported that this interaction between carrier status and aspirin allocation was significant (p = 0.048). Shiffman and colleagues (2009) reported data on the interaction of the LPA rs3798220 variant and aspirin use from the Atherosclerosis Risk in Communities (ARIC) study, a prospective cohort study of risk factors for coronary artery disease in 15,792 individuals. The LPA genetic substudy of ARIC included 6752 individuals with data available for LPA genotype and aspirin use, including 221 individuals with the LPA rs3798220 genotype. Among carriers
of rs3798220, the risk of cardiovascular events was compared in aspirin users and non-users. The HR for non-aspirin users (n = 168) was elevated at 1.57 but did not reach statistical significance (95 % CI: 0.92 to 2.69), while the HR for users of aspirin was not elevated at 0.86 (95 % CI 0.38 to 1.95). Prospective clinical studies are necessary to determine whether selection of persons for chronic aspirin therapy on the basis of rs2798220 status results in improved clinical outcomes.

Area Under the Curve (AUC)-Targeted 5-Fluorouracil Dosing

Diagnostic tests (e.g., Myriad Genetics OnDose) have been developed to measure colorectal cancer patients’ exposure to 5-fluorouracil (5-FU), to help oncologists adjust and optimize 5-FU dosing. This testing is based upon the belief that that 5-FU chemotherapy dosing can be improved by modulating dose to plasma concentration, specifically an AUC target value, as treatment is delivered. An assessment by the BlueCross BlueShield Association Technology Evaluation Center (TEC, 2010) stated that, given the limitations of the existing evidence, the evidence is insufficient to draw conclusions about the impact of 5-FU exposure measurement and AUC-targeted dose adjustment on outcomes of patients administered current chemotherapy regimens for colorectal or head and neck cancer.

To determine if there is adequate evidence that AUC-targeted dose adjustment of 5-FU improves clinical outcomes, the TEC assessment (BCBSA, 2010) focused primarily on the results of 2 randomized, controlled trials, 1 enrolling patients with colorectal cancer (Gamelin et al, 2008) and the other patients with head and neck cancer (Fety et al, 1998).

The randomized controlled trial of AUC-targeted dosing of 5-FU for colorectal cancer reported significantly improved tumor response and a trend toward improved survival using AUC-targeted dosing compared to fixed dosing (Gamelin et al, 2008). The authors of the clinical trial also reported 18 % grade 3 to 4 diarrhea in the fixed-dose arm, which the TEC assessment noted is higher than reported historically, where the rates of grade 3 to 4 diarrhea have ranged from approximately 5 to 7 %. However, the TEC assessment cited an editorial accompanying the study (Wako and McLeod, 2008) that noted that the fixed-dose administration schedule used in this study is "rarely used in current practice in most countries"; the TEC assessment also noted that this administration schedule is absent from current guidelines.

The AUC dose modulation trial in head and neck cancer patients reported overall 5-FU exposures that were significantly reduced after dose adjustment compared to the fixed-dose arm (Fety et al, 1998). This resulted in reduced toxicity, but no improvement in
clinical response. The TEC assessment noted that the dose adjustment method in this trial may have been too complex, as the 12 protocol violations in this treatment arm (of 61 enrolled) were all related to 5-FU dose adjustment mis-calculations (BCBSA, 2010). The TEC assessment stated: "Because patients with protocol violations were not evaluated in an intention-to-treat analysis, the results do not reflect the 'real world' experience of this study." The TEC assessment also noted that the study used a 2-drug induction regimen, which has now been replaced by a 3-drug induction regimen.

**Pharmacogenetic Testing for 5-Fluorouracil Toxicity**

Genetic polymorphisms in the genes coding for dihydropyrimidine dehydrogenase (DPYD) and thymidylate synthase (TYMS), key enzymes in 5-FU metabolism, may result in enzyme products with different activity levels, resulting in 5-FU excess, the accumulation of 5-FU anabolic products, and severe toxicity. TheraGuide 5-FU (Myriad laboratories) is a commercially available test that analyzes DNA from peripheral blood cells to fully sequence the DPYD gene (for the 3 most common DYPD polymorphisms and other rare variants) and detect TYMS variants that increase risk for 5-FU toxicity. An assessment by the BlueCross BlueShield Association Technology Evaluation Center (TEC, 2010) found, however, that testing for genetic variants of the genes coding for DPYD and TYMS enzymes has poor predictive value for 5-FU toxicity and no studies have shown that it is useful in directing 5-FU dose alterations to reduce toxicity without adversely impacting tumor response.

**Mutation Testing in the BRAF V600 Gene**

B-RAF(V600E) (also known as BRAF) kinase mutations occur in about 8% of all solid tumors and approximately 50% to 60% of melanomas; these mutations are found at exon 15, at a single amino acid residue, usually a substitution for valine by glutamic acid, V599E, now referred to as V600E (Nazarian et al, 2010; Dienstmann and Tabernero, 2011). The quantification of BRAF-mutated alleles in plasma may represent a useful biomarker for non-invasive diagnosis and prediction of response to therapy. The development of efficient methods for its detection in free circulating DNA of patients may lead to the improvement of diagnostic and prognostic tools.

Daniotti and colleagues (2007) examined if BRAFV600E represents a detectable marker in the plasma/serum from patients with melanoma. Circulating cell-free DNA was extracted from the serum or plasma of 15 healthy donors and 41 melanoma patients at different clinical stages and obtained either pre-surgery or post-surgery during follow-up. Quantitative analysis showed higher levels of circulating free DNA in patients
compared to controls, with the highest levels detected in samples obtained pre-surgery and at stage IV. Four different PCR methods were compared for their capacity to amplify a few copies of BRAFV600E in wild-type DNA. BRAFV600E was detectable in circulating DNA of 12 patients and in none of the controls; only 1 PCR method reproducibly amplified BRAFV600E. Positive samples were obtained from 8/13 patients at stage IV and from 4/24 patients at stage III, but not in 4 patients at stage I to II; 50 % of the positives were obtained pre-surgery and 50 % at follow-up. Correspondence between circulating DNA and related tumors were examined for 20 patients, and a correlation was found for stage IV patients. The authors concluded that this method can be utilized for monitoring the disease in patients with stage IV melanoma, but it appears unsatisfactory for the early detection of melanoma.

Panka et al (2010) stated that the BRAFV600E mutation has been detected in patients with metastatic melanoma, colon, thyroid and other cancers. Recent studies suggested that tumors with this mutation are especially sensitive to BRAF inhibitors, hence the need to reliably determine the BRAF status of tumor specimens. The present technologies used to screen for this mutation fail to address the problems associated with infiltrating stromal and immune cells bearing wild-type BRAF alleles and thus may fail to detect the presence of mutant BRAFV600E tumors. These investigators developed a rapid, inexpensive method that reduces the contamination of wild-type BRAF sequences from tumor biopsies. The protocol involves a series of PCR amplifications and restriction digestions that take advantage of unique features of both wild-type and mutant BRAF RNA at position 600. Using this protocol, mutant BRAF can be detected in RNA from mixed populations with as few as 0.1 % BRAFV600E mutant cells.

Pinzani and co-workers (2010) proposed an assay based on the use of a locked nucleic acid probe and an allele specific primer to measure plasma-circulating BRAFV600E concentration in patients affected by cutaneous melanoma (n = 55) and non-melanoma skin cancers (n = 13) as well as 18 healthy subjects. The assay is highly sensitive and accurate in detecting down to 0.3 % of mutated allele in plasma. A significant difference between the control group and invasive melanomas (p < 0.01) was evidenced in BRAFV600E concentration, either as relative percentage or absolute values. Receiver operating characteristic curve indicated that BRAFV600E absolute concentration has the maximal diagnostic relevance with 97 % sensitivity and 83 % specificity. Comparison of the results obtained in plasma with those found in the corresponding tissues indicated an 80 % concordance. The authors concluded that the allele specific Taqman-based real-time PCR assay allows the sensitive, accurate and reliable measurement of BRAFV600E mutated DNA in plasma.
Pinzani et al (2011) investigated COLD-PCR (co-amplification at lower denaturation temperature-PCR) as a new approach for the pre-analytical enrichment of the BRAFV600E variant in formalin fixed paraffin embedded (FFPE) melanoma tissues. COLD-PCR was used to selectively amplify BRAFV600E minority alleles from mixtures of wild-type and mutated sequences, and from biological samples. The method showed higher specificity than other conventional PCR-based methods in detecting somatic mutations. These investigators used COLD-PCR to increase the theoretical sensitivity of 3 different post-PCR methods:

I. sequencing,

II. (pyro-sequencing, and

III. (HRMA.

The gain in sensitivity seems to be more evident for HRMA, which allows the detection of 3.1 % mutated alleles. More than 20 % of patients initially classified negative for BRAFV600E were found positive after COLD-PCR. The authors concluded that COLD-PCR was confirmed as a suitable method for the enrichment of mutated alleles, particularly for samples in which the percentage of tumor cells is very low.

On August 17, 2011, the FDA approved vemurafenib (Zelboraf) for the treatment of patients with unresectable or metastatic melanoma with the BRAFV600E mutation as detected by an FDA-approved test. The approval was based on a randomized, open-label trial in patients with previously untreated metastatic or unresectable melanoma with the BRAFV600E mutation as detected by the cobas 4800 BRAF V600 Mutation Test (Roche Molecular Systems, Inc.). This companion diagnostic test was approved by the FDA concurrently with vemurafenib’s approval. The cobas 4800 BRAF V600 test is a PCR-based test. It has several advantages over the commonly used Sanger sequencing, including greater sensitivity and reliability for detecting mutations and quicker results.
On May 29, 2013, the FDA approved 2 new drugs -- dabrafenib (Tafinlar) and trametinib (Mekinist), for patients with metastatic or unresectable melanoma. Tafinlar, a BRAF inhibitor, is approved to treat patients with melanoma whose tumors express the BRAF V600E gene mutation. Mekinist, a MEK inhibitor, is approved to treat patients whose tumors express the BRAF V600E or V600K gene mutations. Approximately 50% of melanomas arising in the skin have a BRAF gene mutation. Tafinlar and Mekinist are being approved as single agents, not as a combination treatment. Furthermore, the FDA approved Tafinlar and Mekinist with a genetic test called the THxID BRAF test, a companion diagnostic that will help determine if a patient’s melanoma cells have the V600E or V600K mutation in the BRAF gene.

On September 4, 2014, the FDA approved pembrolizumab (Keytruda) for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor (e.g., dabrafenib, trametinib, or vemurafenib).

On June 22, 2017, the FDA approved dabrafenib (Tafinlar) and trametinib (Mekinist) administered in combination for patients with metastatic non-small cell lung cancer (NSCLC) with BRAF V600E mutation as detected by an FDA-approved test. These are the first FDA approvals specifically for treatment of patients with BRAF V600E mutation-positive metastatic NSCLC. Furthermore, the FDA approved a next generation sequencing (NGS) test called Oncomine™ Dx Target Test, a companion diagnostic that will help detect multiple gene mutations for lung cancer in a single test from a single tissue specimen. This test detects the presence of BRAF, ROS1, and EGFR gene mutations or alterations in tumor tissue of patients with NSCLC. This test can be used to select patients with NSCLC with the BRAF V600E mutation for treatment with the combination of dabrafenib and trametinib. This is the first NGS oncology panel test approved by the FDA for multiple companion diagnostic indications.

The approvals are based on Study BRF113928 (NCT01336634), an international, multicenter, three-cohort, non-randomized, non-comparative, open-label, trial in patients with locally confirmed BRAF V600E mutation-positive metastatic NSCLC. Ninety-three patients were treated with the combination of dabrafenib (150 mg orally twice daily) and trametinib (2 mg orally once daily). Of these 93 patients, 36 had received no prior systemic therapy for metastatic NSCLC and 57 received at least one platinum-based chemotherapy regimen with demonstrated disease progression. Seventy-eight patients with previously treated BRAF V600E mutation-positive NSCLC received single-agent dabrafenib. In the previously treated group, the overall response rate (ORR) for the combination based on independent radiology review committee assessment per RECIST 1.1 was 63% (95% CI: 49%, 76%) with a median duration of
response (DoR) of 12.6 months (95% CI: 5.8, not estimable [NE]). In the treatment-naive group, the ORR for the combination was 61% (95% CI: 44%, 77%) and median DoR was not estimable (95% CI: 6.9, NE); however, 59% of responders had response durations greater than six months. The ORR for patients who received single-agent dabrafenib was 27% (95% CI: 18%, 38%) and the median DoR was 9.9 months. The incidence and severity of adverse reactions occurring in patients with NSCLC were generally similar to those reported in prior approvals for patients with melanoma. The most common adverse reactions (≥20%) were pyrexia, fatigue, nausea, vomiting, diarrhea, dry skin, decreased appetite, edema, rash, chills, hemorrhage, cough, and dyspnea. The most common Grade 3-4 adverse reactions were pyrexia, fatigue, dyspnea, vomiting, rash, hemorrhage, and diarrhea. The majority of laboratory abnormalities were Grade 1-2. The most common (≥5%) Grade 3-4 laboratory abnormalities were hyponatremia, lymphopenia, anemia, hyperglycemia, neutropenia, leukopenia, hypophosphatemia, and increased alanine aminotransferase. Dabrafenib and trametinib were discontinued for adverse reactions in 18% and 19% of patients, respectively. The recommended doses are dabrafenib 150 mg orally twice daily, approximately 12 hours apart, with trametinib 2 mg orally once daily. The presence of BRAF V600E mutation in tumor specimen should be confirmed by an FDA-approved test prior to initiation of therapy.

On May 4, 2018, the FDA approved Tafinlar (dabrafenib) and Mekinist (trametinib), administered together, for the treatment of anaplastic thyroid cancer (ATC) that cannot be removed by surgery or has spread to other parts of the body (metastatic), and has a type of abnormal gene, BRAF V600E (BRAF V600E mutation-positive).

The efficacy of Tafinlar and Mekinist in treating ATC was shown in an open-label clinical trial of patients with rare cancers with the BRAF V600E mutation. Data from trials in BRAF V600E mutation-positive, metastatic melanoma or lung cancer and results in other BRAF V600E mutation-positive rare cancers provided confidence in the results seen in patients with ATC. The trial measured the percent of patients with a complete or partial reduction in tumor size (overall response rate). Of 23 evaluable patients, 57 percent experienced a partial response and 4 percent experienced a complete response; in nine (64 percent) of the 14 patients with responses, there were no significant tumor growths for six months or longer. The side effects of Tafinlar and Mekinist in patients with ATC are consistent with those seen in other cancers when the two drugs are used together. Common side effects include fever (pyrexia), rash, chills, headache, joint pain (arthralgia), cough, fatigue, nausea, vomiting, diarrhea, myalgia (muscle pain), dry skin, decreased appetite, edema, hemorrhage, high blood pressure (hypertension) and difficulty breathing (dyspnea). Severe side effects of Tafinlar include the development of new cancers, growth of tumors in patients with BRAF wild-type tumors, serious bleeding problems, heart problems, severe eye problems, fever that may be severe, serious skin
reactions, high blood sugar or worsening diabetes, and serious anemia. Severe side effects of Mekinist include the development of new cancers; serious bleeding problems; inflammation of intestines and perforation of the intestines; blood clots in the arms, legs or lungs; heart problems; severe eye problems; lung or breathing problems; fever that may be severe; serious skin reactions; and high blood sugar or worsening diabetes. Both Tafinlar and Mekinist can cause harm to a developing fetus; women should be advised of the potential risk to the fetus and to use effective contraception.

On June 27, 2018, the FDA approved encorafenib and encorafenib (Braftovi) in combination with binimetinib (Mektovi) for patients with unresectable or metastatic melanoma with a BRAF V600E or V600K mutation, as detected by an FDA-approved test. The recommended doses are binimetinib 45 mg orally twice daily and encorafenib 450 mg orally once daily. Furthermore, the FDA also granted approval of the THxID BRAF Kit (bioMérieux) as a companion diagnostic for this therapy.

Approval for combination therapy with Braftovi and Mektovi was based on a randomized, active-controlled, open-label, multicenter trial (COLUMBUS; NCT01909453) in 577 patients with Braf V600E or V600K mutation-positive unresectable or metastatic melanoma. Patients were randomized (1:1:1) to receive binimetinib 45 mg twice daily plus encorafenib 450 mg once daily, encorafenib 300 mg once daily, or vemurafenib 960 mg twice daily. Treatment continued until disease progression or unacceptable toxicity. The major efficacy measure was progression-free survival (PFS) using RECIST 1.1 response criteria and assessed by blinded independent central review. The median PFS was 14.9 months for patients receiving binimetinib plus encorafenib, and 7.3 months for the vemurafenib monotherapy arm (hazard ratio 0.54, 95% CI: 0.41, 0.71, p<0.0001). Overall response rates assessed by central review were 63% and 40%, respectively. Median response duration was 16.6 months vs. 12.3 months, respectively. The most common (>25%) adverse reactions in patients receiving the combination were fatigue, nausea, diarrhea, vomiting, abdominal pain, and arthralgia. Discontinuation of therapy due to adverse reactions occurred in 5% of patients receiving the combination; the most common reasons were hemorrhage and headache.

**IL28B Polymorphism Genotyping for Interferon Therapy for Hepatitis C**

The IL28B gene is involved with viral resistance and is up-regulated by interferons. IL28B polymorphisms appear to be a strong independent predictor of viral responsiveness. Thus, it is likely that testing for IL28B polymorphisms will be included in making treatment decisions and potentially guiding therapy. Currently, there are no studies evaluating the impact of IL28B on treatment decisions. Well-designed
studies are needed to clarify the role, if any, of IL28B polymorphism genotyping for interferon therapy for hepatitis C.

**CYP2D6 Polymorphism and Alzheimer’s Disease**

In a multi-center, prospective cohort study, Pilotto et al (2009) evaluated the influence of the single nucleotide polymorphism rs1080985 in the cytochrome P450 2D6 (CYP2D6) gene on the efficacy of donepezil in patients with mild to moderate Alzheimer’s disease (AD). A total of 127 white patients with AD according to the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer’s Disease and Related Disorders Association Work Group criteria were included in this study. Patients were treated with donepezil 5 to 10 mg/daily for 6 months. Cognitive and functional statuses were evaluated at baseline and at 6-month follow-up. Response to therapy was defined according to the National Institute for Health and Clinical Excellence criteria. Compliance and drug-related adverse events were also evaluated. The analyses identifying the CYP2D6 and APOE polymorphisms were performed in blinded fashion. At 6-month follow-up, 69 of 115 patients (60 %) were responders and 46 patients (40 %) were non-responders to donepezil treatment. A significantly higher frequency of patients with the G allele of rs1080985 was found in non-responders than in responders (58.7 % versus 34.8 %, p = 0.013). Logistic regression analysis adjusted for age, sex, Mini-Mental State Examination score at baseline, and APOE demonstrated that patients with the G allele had a significantly higher risk of poor response to donepezil treatment (odds ratio 3.431, 95 % CI: 1.490 to 7.901). The authors concluded that the single nucleotide polymorphism rs1080985 in the CYP2D6 gene may influence the clinical efficacy of donepezil in patients with mild to moderate AD. The analysis of CYP2D6 genotypes may be useful in identifying subgroups of patients with AD who have different clinical responses to donepezil.

In a multi-center prospective cohort study, Seripa et al (2011) evaluated the effect of 16 functional polymorphisms in the CYP2D6 gene on the clinical response to donepezil treatment in patients with mild-to-moderate AD. These researchers evaluated 57 unrelated Caucasians clinically diagnosed as AD according to the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer’s Disease and Related Disorders Association Work Group criteria. Patients were treated with donepezil (5 to 10 mg/daily) for 6 months. The response to donepezil treatment was evaluated at 6-month follow-up according to the National Institute for Health and Clinical Excellence requirements. The identification of 16 clinically relevant CYP2D6 gene variants was performed by a high-throughput genetic analysis. Thirty-eight of 57 patients (67 %) were responders and 19 patients (33 %) were non-responders to donepezil treatment. A
significantly higher frequency of gene variants conferring decreased or absent enzyme activity was observed in responder than in non-responder patients (73.68 % versus 36.84 %; p = 0.005). The presence of gene variants conferring decreased or absent activity of the CYP2D6 enzyme was significantly associated with a clinical response to donepezil treatment (odds ratio = 6.286; 95 % CI: 1.828 to 21.667). The authors concluded that functional polymorphisms in the CYP2D6 gene can influence the clinical efficacy of donepezil. The analysis of CYP2D6 genotypes may be useful in identifying subgroups of AD patients with different clinical response to donepezil treatment.

The Vysis ALK Break Apart FISH Probe Kit

Crizotinib (Xalkori) is a kinase inhibitor which has recently received accelerated FDA approval for use in locally advanced or metastatic NSCLC that is ALK-positive as detected by an FDA-approved test. The FDA approved the Vysis ALK Break Apart FISH Probe Kit concurrently with crizotinib as a companion diagnostic test designed to detect rearrangements of the ALK gene in NSCLC. FISH refers to fluorescence in-situ hybridization, which has numerous uses, including identify whether too many, or too few, copies of a particular gene are present in the body’s cells or whether certain genes have rearrangements that play an active role in disease progression (Abbott Press Release, 2011).

Oncogenic fusion genes consisting of EML4 and anaplastic lymphoma kinase (ALK) are present in a subgroup of non-small-cell lung cancers, representing 2 to 7 % of such tumors (Kwak et al, 2010). Rodig et al (2010) noted that “crizotinib has been particularly effective against anaplastic large cell lymphoma and non-small cell lung cancer (NSCLC) cell lines that harbor ALK translocations resulting in expression of oncogenic ALK fusion proteins”.

Kwak et al (2011) screened tumor samples from approximately 1500 NSCLC patients for the presence of ALK rearrangements and found 82 patients with advanced ALK-positive disease in their study population. These patients were subsequently enrolled in a clinical trial of crizotinib therapy. At a mean treatment duration of 6.4 months, the overall response rate was 57 %, with an estimated probability of 6-month progression free survival was estimated at study completion to be 72 %. The investigators also noted patients with ALK rearrangements tended to be younger than those without, and most of these patients were light or non-smokers.

The 2010 American Society of Clinical Oncology Annual Meeting included a presentation on crizotinib for the treatment of advanced NSCLC. Stinchcombe et al
(2011) summarized the evidence presented and stated that the results of crizotinib in molecularly selected patients with advanced NSCLC whose tumor cells had a novel fusion protein involving ALK reinforces the importance of understanding molecular heterogeneity in this patient population.

**GeneSightRx Testing**

GeneSightRx, developed by AssureRx Health (Mason, OH), is a technology that measures and analyzes genomic variants affecting the metabolism and response to health medications in patients. These genomic tests were developed to serve as a clinical treatment support tool, supposedly providing objective genetic-based patient information in advance of making a medication decision. Knowing a patient's genetic profile may aid in understanding which medications the patient would metabolize properly and help inform treatment choices unique to each patient. GeneSightRx Psychotropic analyzes 6 genes that may affect a patient’s response to anti-depressant and anti-psychotic medications. Four pharmacokinetic genes from the cytochrome P450 family and 2 pharmacodynamic genes related specifically to the serotonin system are genotyped.

Hall-Flavin et al (2013) reported on an open-label study to evaluate the potential benefit of GeneSight for the management of psychotropic medications used to treat major depression in an outpatient psychiatric practice. The open-label study was divided into two groups. In the first (unguided) group (n = 113), pharmacogenomic information was not shared until all participants completed the study. In the second (guided) group (n = 114), the pharmacogenomic report was provided to physicians for clinical use. Three depression ratings, the 17-item Hamilton Rating Scale for Depression (HAMD-17), the Quick Inventory of Depressive Symptomatology - Clinician Rated (QIDS-C16), and the Patient Health Questionnaire (PHQ-9), were collected at baseline, and at 2, 4, and 8 weeks. The guided group experienced greater percent improvement in depression scores from baseline on all three depression instruments (HAMD-17, P < 0.0001; QIDS-C16, P < 0.0001; PHQ-9, P < 0.0001) compared with the unguided group. Eight-week response rates were higher in the guided group than in the unguided group on all three measurements (HAMD-17, P = 0.03; QIDS-C16, P = 0.005; PHQ-9, P = 0.01). Eight-week QIDS-C16 remission rates were higher in the guided group (P = 0.03). Participants in the unguided group who at baseline were prescribed a medication that was most discordant with their genotype experienced the least improvement compared with other unguided participants (HAMD-17, P = 0.007). Participants in the guided group and on a baseline medication most discordant with their genotype showed the greatest improvement compared with the unguided cohort participants (HAMD-17, P = 0.01).
Winner et al (2013) reported on a prospective double-blind randomized control trial (RCT) to evaluate the benefit of a combinatorial, five gene pharmacogenomic test and interpretive report (GeneSight) for the management of psychotropic medications used in the treatment of major depression in an outpatient psychiatric practice. Depressed adult outpatients were randomized to a treatment as usual (TAU, n=25) arm or a pharmacogenomic-informed GeneSight (n=26) arm. Subjects were blinded to their treatment group and depression severity was assessed by blinded study raters. Within two days of enrollment, clinicians of subjects in the guided group received the GeneSight report that categorized each of 26 psychotropic medications within a green, yellow, or red “bin” based on the relationship of each medication to a subject’s pharmacokinetic and pharmacodynamic combinatorial gene variant profile. Antidepressant medication changes began within 2 weeks after baseline assessments. Depression severity was assessed by blinded study raters using the HAMD-17, PHQ-9, QIDS-SR, and QIDS-CR administered 4, 6, and 10 weeks after baseline assessment. Between-group nonsignificant trends were observed with greater than double the likelihood of response and remission in the GeneSight group measured by HAMD-17 at week 10. Mean percent improvement in depressive symptoms on HAMD-17 was higher for the GeneSight group over TAU (30.8% vs 20.7%; p = 0.28), although the difference was not statistically significant. TAU subjects who had been prescribed medications at baseline that were contraindicated based on the individual subject’s genotype (i.e., red bin) had less improvement (0.8%) in depressive symptoms measured by HAMD-17 at week 10 than the pharmacogenomics guided subjects who started on a red bin medication (33.1%, p = 0.06) and the improvement in GeneSight subjects overall (26.4%, p=0.08), although differences were not statistically significant.

Winner et al (2013) reported on a 1 year blinded and retrospective study that evaluated eight direct or indirect health care utilization measures for 96 patients with a DSM-IV-TR diagnosis of depressive or anxiety disorder. The eight measures were evaluated in relation Genesight, an interpretive pharmacogenomic test and reporting system, designed to predict antidepressant responses based on DNA variations in cytochrome P450 genes (CYP2D6, CYP2C19, CYP2C9 and CYP1A2), the serotonin transporter gene (SLC6A4) and the serotonin 2A receptor gene (5HTR2A). All subjects had been prescribed at least one of 26 commonly prescribed antidepressant or antipsychotic medications. Subjects whose medication regimen included a medication identified by the gene-based interpretive report as most problematic for that patient and are in the 'red bin' (medication status of 'use with caution and frequent monitoring'), had 69% more total health care visits, 67% more general medical visits, greater than three-fold more medical absence days, and greater than four-fold more disability claims than subjects taking drugs categorized by the report as in the green bin ('use as directed') or
yellow bin (‘use with caution’). There were no correlations between the number of medications taken and any of the eight healthcare utilization measures.

Kirchheiner et al (2010) stated that more than 50 years of pharmacogenetic research have produced many examples of the impact of inherited variability in the response to psychotropic drugs. These successes, however, have as yet failed to translate into broadly applicable strategies for the improvement of individual drug treatment in psychiatry. One important argument against the widespread adoption of pharmacogenetics as a clinical tool is the lack of evidence showing its impact on medical decision making and on risk benefit ratio for the patients. The individual drug metabolizing capacity is assessed by genotyping drug metabolizing enzymes. The potential implications of information gained from genotyping are dose adjustments according to genotype. However, even when the consequences of genotype on pharmacokinetics are significant and well known, as in the case of many tricyclic antidepressants and several selective serotonin reuptake inhibitors (SSRIs), there is still considerable controversy on whether adjustment of dosage driven by genetic information may improve therapeutic efficacy, and/or adverse events is prevented, to an extent of any practical importance in clinical practice. Different types of pharmacogenetic studies may improve the understanding of the functional consequence of a genetic variant in the clinical setting. The use of intermediate phenotypes instead of broad outcome parameters such as drug response or remission might improve the knowledge on what exactly happens if an individual with a specific genotype takes a certain drug.

Fleeman et al (2010) examined if testing for cytochrome P450 (CYP) polymorphisms in adults entering anti-psychotic treatment for schizophrenia leads to improvement in outcomes, is useful in medical, personal or public health decision-making, and is a cost-effective use of health-care resources. The following electronic databases were searched for relevant published literature: Cochrane Controlled Trials Register, Cochrane Database of Systematic Reviews, Database of Abstracts of Reviews of Effectiveness, EMBASE, Health Technology Assessment database, ISI Web of Knowledge, MEDLINE, PsycINFO, NHS Economic Evaluation Database, Health Economic Evaluation Database, Cost-effectiveness Analysis (CEA) Registry and the Centre for Health Economics website. In addition, publicly available information on various genotyping tests was sought from the internet and advisory panel members. A systematic review of analytical validity, clinical validity and clinical utility of CYP testing was undertaken. Data were extracted into structured tables and narratively discussed, and meta-analysis was undertaken when possible. A review of economic evaluations of CYP testing in psychiatry and a review of economic models related to schizophrenia were also carried out. For analytical validity, 46 studies of a range of different genotyping tests for 11
different CYP polymorphisms (most commonly CYP2D6) were included. Sensitivity and specificity were high (99 to 100 %). For clinical validity, 51 studies were found. In patients tested for CYP2D6, an association between genotype and tardive dyskinesia (including Abnormal Involuntary Movement Scale scores) was found. The only other significant finding linked the CYP2D6 genotype to parkinsonism. One small unpublished study met the inclusion criteria for clinical utility. One economic evaluation assessing the costs and benefits of CYP testing for prescribing anti-depressants and 28 economic models of schizophrenia were identified; none was suitable for developing a model to examine the cost-effectiveness of CYP testing. The authors concluded that tests for determining genotypes appear to be accurate although not all aspects of analytical validity were reported. Given the absence of convincing evidence from clinical validity studies, the lack of clinical utility and economic studies, and the unsuitability of published schizophrenia models, no model was developed; instead key features and data requirements for economic modeling were presented. Recommendations for future research cover both aspects of research quality and data that will be required to inform the development of future economic models.

Lohoff and Ferraro (2010) noted that despite continued efforts to optimize pharmacological treatment for individuals with psychiatric disorders, efficacy and tolerability of medication remains highly variable. In addition to clinical heterogeneity, diagnostic uncertainty, environmental, social factors, and genetic factors have been identified as playing an important role in the inter-individual differences in therapeutic and toxic drug effects. This article described recent developments in the field of psychiatric pharmacogenetics and focused on anti-depressant, anti-psychotic, anti-convulsant, and mood-stabilizing drugs. Recent findings from pharmacogenetic clinical trials were reviewed and clinical implications were discussed. The authors concluded that although current data are too sparse to allow the development of guidelines for using pharmacogenetic testing in routine clinical practice, the field of psychiatric pharmacogenetics is rapidly developing and identification of genetic biomarkers that predict therapeutic response and risk of side effects will ultimately help the practitioner to choose effective and safe treatment for patients suffering from psychiatric disorders.

Plesnicar (2010) stated that anti-psychotics are the lodestar in the treatment of schizophrenia despite the variability of the therapeutic response and drug-induced adverse effects (especially extrapyramidal symptoms, gain weight, and metabolic disturbances). More and more data are supporting the notion that genetic factors -- as well as often over-looked personal and environmental factors -- that define the inter-individual differences in pharmacokinetic and pharmacodynamic treatment response. At present, there are no practical pharmacogenetic tests that could be used in everyday clinical practice; however, in the field of psychiatry they are expected within a few
years. Pharmacogenetic tests will indubitably become an important tool for personalized prescription.

Gelenberg (2010) noted that variety of American and European guidelines are available for clinicians treating major depressive disorder and depressive subtypes. Major Western guidelines published since 2000 made similar recommendations for all stages of treatment for depression, including a reliance on measurement-based care. First-line treatment is usually a SSRI, psychotherapy, or a combination of pharmacotherapy and psychotherapy. Next-step treatment recommendations are switching or augmentation, depending on patient response to the initial treatment. Maintenance therapy continues the approach that led to remission. The American Psychiatric Association will release a new treatment guideline to offer information on developments made since the last guidelines were published in 2000. Despite progress made during the last decade, no major breakthroughs in the treatment of depression have occurred, and genetic testing developments allowing for personalized care remain the goal of research.

Lewis et al (2011) tested the hypothesis that patients homozygous for the long (insertion) polymorphism of the serotonin transporter (5-HTTLPR) have an increased response to SSRI antidepressants but not to noradrenaline reuptake inhibitors (NARIs) antidepressants. In an individually randomized, parallel-group controlled trial, people meeting criteria for a depressive episode who were referred by their general practitioner were randomized to receive either citalopram (an SSRI) or reboxetine (an NARI). Randomization was by means of a remote automated system accessed by telephone. The main outcome was depressive symptoms, measured by Beck Depression Inventory (BDI) total score 6 weeks after randomization. A total of 298 participants were randomized to receive citalopram and 303 were randomized to reboxetine. At 6 weeks follow-up, complete data were available for 258 participants taking citalopram and 262 taking reboxetine. These investigators found no evidence to support an influence of 5-HTTLPR on outcome following antidepressant treatment. The interaction term for BDI score at 6 weeks was 0.50 (95 % CI: -2.04 to 3.03, p = 0.70), which indicated that responses to the SSRI and NARI were similar irrespective of 5-HTTLPR genotype. The authors concluded that it is unlikely that the 5-HTTLPR polymorphism alone will be clinically useful in predicting response to antidepressants in people with depression.

In a review and meta-analysis, Biernacka et al (2012) evaluated the evidence for association between the serotonin transporter gene promoter polymorphism (5HTTLPR) and antidepressant induced mania (AIM). Medline up to November 2009 was searched for key words bipolar, antidepressant, serotonin transporter, SLC6A4, switch, and mania. A total of 5 studies have evaluated the SLC6A4 promoter polymorphism and AIM in adults (total n = 340 AIM+ cases, n = 543 AIM- controls). Although a random
effects meta-analysis showed weak evidence of association of the S allele with AIM+ status, a test of heterogeneity indicated significant differences in estimated genetic effects between studies. A similar weak association was observed in a meta-analysis based on a subset of 3 studies that excluded patients on mood stabilizers; however the result was again not statistically significant. The authors concluded that there is insufficient published data to confirm an association between 5HTTLPR and antidepressant induced mania. Pharmacogenomic studies of antidepressant induced mania have high potential clinical impact provided future studies are of adequate sample size and include rigorously assessed patient characteristics (e.g., ancestry, rapid cycling, concurrent mood stabilization, and length of antidepressant exposure).

Vetti et al (2010) systematically categorized the experience from routine CYP2D6 genotyping in a diagnostic laboratory. All samples submitted to the authors' laboratory for CYP2D6 genotyping in the period of June 29, 1998 to December 28, 2009 were examined retrospectively. The samples were classified into 3 indication groups based on clinical information given in the request form. All samples, and a control group consisting of 100 healthy blood donors, were tested for the 4 most prevalent non-functional CYP2D6 alleles in the European population, and for ultra-rapid metabolizer-associated duplications of the gene. A total of 325 samples were included. The proportion of ultra-rapid metabolizers was significantly higher in the patient group (4.0 %, \( p = 0.045 \)) than in the control group (0 %), with the highest proportion among those patients that used a known CYP2D6 substrate. The percentage of poor metabolizers was not significantly higher in the patient group (8.3 %) than in the control group (6.0 %) (\( p = 0.528 \)). The authors concluded that the CYP2D6 analysis could rarely explain the patients' side effects or lack of drug response, even though the study group was selected because of clinical problems due to drugs they were using. Two explanations may be that the indication(s) for genetic testing is not clearly defined and that the CYP2D6 genotype is only one of many factors that determine individual drug response.

Jurgens et al (2012) examined the clinical impact of CYP2D6 genotype in patients with a diagnosis within the schizophrenic spectrum using medication pattern as proxy for therapeutic and side effect. The study was conducted in patients genotyped during an inpatient stay (\( n = 576 \)). Continuous antipsychotic, adjuvant, and anticholinergic drug regimens were registered retrospectively in a cross-sectional manner before genotyping. Antipsychotics were divided into CYP2D6 dependent and independent, and dose equivalents were calculated as chlorpromazine equivalents (CPZEq). Poor metabolizers and ultra-rapid metabolizers were treated with significantly higher median CPZEq doses (625.8; inter-quartile range [IQR] 460.4 to 926.7; and 550; IQR, 199.8 to 1,049) than extensive metabolizers (EMs) and intermediate metabolizers (IMs) (384; IQR, 150 to 698; and 446; IQR, 150 to 800) (\( p = 0.018 \)). Logistic regression showed no
association between anticholinergic treatment and CYP2D6 genotype or concomitant treatment with CYP2D6 inhibitors (p = 0.79 and p = 0.46, respectively). The authors concluded that these findings indicated that CYP2D6 genotype has no sufficient clinical impact that poor metabolizers and ultra-rapid metabolizers are easily clinically identified with.

Altar et al (2015) collected DNA of 258 patients with treatment-resistant depression in three 8-10 week, two-arm, prospective clinical trials. Forty-four allelic variations were measured in genes for the cytochrome P450 (CYP) enzymes CYP2D6, CYP1A2, the serotonin transporter (SLC6A4), and the 5-HT2A receptor (HTR2A). The combinatorial pharmacogenomic (CPGx™) GeneSight test results were provided to clinicians to support medication changes from baseline (guided arm), or they were provided at the end of each study to clinicians of unguided patients who were treated as usual (TAU). TAU subjects who at baseline were prescribed medications genetically discordant for them showed only a 12% symptom improvement, far less than the 32.5% or 28.5% improvements of the TAU subjects on yellow-category ('use with caution'; p = 0.002) or green-category medications ('use as recommended'; p = 0.02), respectively. The odds of a clinical response were increased 2.3-fold among all GeneSight-guided compared to all TAU subjects (p = 0.004), and overall, the guided group had a 53% greater improvement in depressive symptoms (p = 0.0002), a 1.7-fold relative improvement in response (p = 0.01), and a number needed to treat for one clinical response above that seen in the TAU group of 6.07. However, lack of blinding in two of the three studies limits the validity of the meta-analysis.

Greden, et al. (2019) reported on the outcomes of the GUIDED study, where outpatients (N = 1167) diagnosed with MDD and with a patient- or clinician-reported inadequate response to at least one antidepressant were enrolled in the Genomics Used to Improve DEpression Decisions (GUIDED) trial – a rater- and patient-blind randomized controlled trial. Patients were randomized to treatment as usual (TAU) or a pharmacogenomics-guided intervention arm in which clinicians had access to a pharmacogenomic test report to inform medication selections (guided-care). Medications were considered congruent ('use as directed' or 'use with caution' test categories) or incongruent ('use with increased caution and with more frequent monitoring' test category) with test results. Unblinding occurred after week 8. Primary outcome was symptom improvement [change in 17-item Hamilton Depression Rating Scale (HAM-D17)] at week 8; secondary outcomes were response (≥50% decrease in HAM-D17) and remission (HAM-D17 ≤ 7) at week 8. At week 8, symptom improvement for guided-care was not significantly different than TAU (27.2% versus 24.4%, p = 0.107); however, improvements in response (26.0% versus 19.9%, p = 0.013) and remission (15.3% versus 10.1%, p = 0.007) were statistically significant. Patients taking incongruent medications prior to baseline who
switched to congruent medications by week 8 experienced greater symptom improvement (33.5% versus 21.1%, p = 0.002), response (28.5% versus 16.7%, p = 0.036), and remission (21.5% versus 8.5%, p = 0.007) compared to those remaining incongruent. The investigators concluded that pharmacogenomic testing did not significantly improve mean symptoms but did significantly improve response and remission rates for difficult-to-treat depression patients over standard of care.

The GUIDED study failed to achieve statistical significance in the prespecified primary outcome, change in 17-item Hamilton Depression Rating Scale (HAM-D17) at week 8. Some secondary endpoints achieved statistical significance, specifically response (≥50% decrease in HAM-D17) and remission (HAM-D17 ≤ 7) rates at week 8. However, the study had 25 secondary outcome measures and 3 “other” outcome measures. Although it is possible to design a trial to have more than one primary endpoint, those primary endpoints would need to be prespecified, and the statistical analysis adjusted to account for multiple primary endpoints (multiplicity). In a post hoc reanalysis of data, statistical significance in the primary HAM-D17 endpoint was achieved if the analysis only included participants who were taking medications subject to gene-drug interactions at baseline. Although it is possible to design a study that focused the analysis on these subjects who were taking medications subject to gene-drug interactions, this would have to be prespecified in order to avoid the potential bias from after-the-fact data reanalysis.

There are other concerns about the GUIDED study. The study compared GeneSight guided therapy to “treatment as usual.” It is not known whether similar improvements in outcomes could be achieved by providing the clinicians with information about known drug-drug interactions and side-effect profile of the various antidepressants, as well as other information relevant to selection of antidepressants (minus the pharmacogenetic test results). This is especially relevant given that the study was not limited to psychiatrists, but included primary care physicians. Another issue relates to the magnitude of the benefit from testing; a letter to the editor questioned the clinical significance of the reported improvements in the secondary outcomes.

**GeneCept**

Brennan et al (2015) examined the effectiveness of genetic testing in a real-world setting and to assess its impact on clinician treatment decisions. This was a naturalistic, unblinded, prospective analysis of psychiatric patients and clinicians who utilized a commercially available genetic test (between April and October of 2013), which incorporates 10 genes related to pharmacokinetics and pharmacodynamics of psychiatric medications. Each patient’s genetic results were provided to participating
clinicians, who completed a baseline survey including patient medications, history, and severity of illness. Clinicians were prompted to complete surveys within 1 week of receiving the genetic results and again 3 months later. Patients likewise completed assessments of depression, anxiety, medication side effects, and quality of life at baseline, 1 month, and 3 months. Data from 685 patients were collected. Approximately 70% and 29% of patients had primary diagnoses of either a mood or anxiety disorder, respectively. Clinician-reported data, as measured by the Clinical Global Impressions-Improvement scale, indicated that 87% of patients showed clinically measurable improvement (rated as very much improved, much improved, or minimally improved), with 62% demonstrating clinically significant improvement. When analysis was restricted to the 69% of individuals with ≥ 2 prior treatment failures, 91% showed clinically measurable improvement. Patients also reported significant decreases in depression (P < .001), anxiety (P < .001), and medication side effects (P < .001) and increases in quality of life (P < .001). The authors concluded that these results suggest that a substantial proportion of individuals receiving pharmacogenetic testing showed clinically significant improvements on multiple measures of symptoms, adverse effects, and quality of life over 3 months. In the absence of randomization, blinding, and a control group, the study does not provide a rigorous assessment of the GeneCept assay’s ability to improve symptom outcomes.

IDgenetix

Olson et al (2017) stated pharmacogenetic testing holds promise as a personalized medicine tool by permitting individualization of pharmacotherapy in accordance with genes influencing therapeutic response, side effects, and adverse events. The authors evaluated the effect on outcomes for patients diagnosed with neuropsychiatric disorders of pharmacogenetics (PGx)-guided treatment compared to usual standard of care. This was a prospective, randomized study of 237 patients at an outpatient community-based psychiatric practice conducted between April 2015 and October 2015. Baseline patient assessments and a buccal swab were collected for pharmacogenetic testing at study initiation. For the experimental group, PGx results were provided to the clinicians as guides to treatment. Control subjects were treated according to the usual standard of care with no clinician reference to their PGx results. Neuropsychiatric Questionnaire (NPQ) and Symbol Digit Coding Test (SDC) scores and adverse drug events, hospitalizations, and medication information were collected at 30, 60, and 90 days. More than half (53%) of patients in the control group reported at least 1 adverse drug event compared to 28% of patients with PGx-guided medication management (P = .001). NPQ and SDC scores improved for both groups, but no statistical difference in efficacy as measured by these assessments was observed within the 90-day observation
period. The authors concluded that pharmacogenetic testing may facilitate psychiatric drug therapy with greater tolerability and similar efficacy compared to standard of care.

Sugarman et al (2016) stated among long-term care facility residents, polypharmacy is common, and often appropriate, given the need to treat multiple, complex, chronic conditions. Polypharmacy has, however, been associated with increased healthcare costs, adverse drug events, and drug interactions. The current study evaluates the potential medication cost savings of adding personalized pharmacogenetic information to traditional medication management strategies. One hundred and twelve long-term care residents completed pharmacogenetic testing for targeted variants in the following genes: CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4/CYP3A5, HTR2A, HTR2C, SLC6A4, SLC6A2 COMT, OPRM1, SLCO1B1, VKORC1 and MTHFR. Following reporting of the IDgenetix Polypharmacy® test results, an internal medication management assessment was performed by a licensed clinical pharmacist to identify potential opportunities for regimen optimization through medication changes or discontinuations. The medication cost differences before and after the pharmacogenetic-guided review were assessed. Medication review following pharmacogenetic result reporting identified 54 patients (48.2%) with a total of 132 drug change recommendations (45 reductions; 87 replacements) and an average of 2.4 proposed medication changes (range 1-6) per patient. Medication cost savings related to the identified reduction and replacement opportunities exceeded the cost of testing and are estimated to be US$ 1300 (year 2016 cost) per patient annually assuming full implementation. The authors concluded that compared with traditional medication review, pharmacogenetic testing resulted in a 38% increase in the number of patients with current medication change opportunities and also offered valuable genetic information that could be referenced to personalize future prescribing decisions for all patients.

**ABCB1 gene**

In a small pilot study, Breitenstein et al (2014) stated the gene product of the ABCB1 gene, the P-glycoprotein, functions as a custodian molecule in the blood-brain barrier and regulates the access of most antidepressants into the brain. Previous studies showed that ABCB1 polymorphisms predicted the response to antidepressants that are substrates of the P-gp, while the response to non-substrates was not influenced by ABCB1 polymorphisms. The aim of the present study was to evaluate the clinical application of ABCB1 genotyping in antidepressant pharmacotherapy. Data came from 58 depressed inpatients participating in the Munich Antidepressant Response Signature (MARS) project, whose ABCB1 gene test results were implemented into the clinical decision making process. Hamilton Depression Rating Scale (HAM-D) scores, remission
rates, and duration of hospital stay were documented with dose and kind of antidepressant treatment. Patients who received ABCB1 genotyping had higher remission rates \( \chi^2(1) = 6.596, p = 0.005, \text{1-sided} \) and lower Hamilton sores \( t(111) = 2.091, p = 0.0195, \text{1-sided} \) at the time of discharge from hospital as compared to patients without ABCB1 testing. Among major allele homozygotes for ABCB1 single nucleotide polymorphisms (SNPs) rs2032583 and rs2235015 (TT/GG genotype), an increase in dose was associated with a shorter duration of hospital stay \( \rho(28) = -0.441, p = 0.009, \text{1-sided} \), whereas other treatment strategies (e.g., switching to a non-substrate) showed no significant associations with better treatment outcome. Discussion The implementation of ABCB1 genotyping as a diagnostic tool influenced clinical decisions and led to an improvement of treatment outcome. Patients carrying the TT/GG genotype seemed to benefit from an increase in P-gp substrate dose. The authors concluded that results suggest that antidepressant treatment of depression can be optimized by the clinical application of ABCB1 genotyping.

BRCA Testing for Olaparib (Lynparza)

The FDA approved BRACAnalysis CDx (Myriad Genetics) as a companion diagnostic for olaparib (Lynparza) (AstraZeneca), a drug for that has been approved by the FDA for women with advanced ovarian cancer associated with BRCA genes.

BRACAnalysis CDx is an in vitro diagnostic device intended for the qualitative detection and classification of variants in the protein coding regions and intron/exon boundaries of the BRCA1 and BRCA2 genes using genomic DNA obtained from whole blood specimens collected in EDTA (FDA, 2014). Single nucleotide variants and small insertions and deletions (indels) are identified by polymerase chain reaction (PCR) and Sanger sequencing. Large deletions and duplications in BRCA1 and BRCA2 are detected using multiplex PCR. Results of the test are used as an aid in identifying ovarian cancer patients with deleterious or suspected deleterious germline BRCA variants eligible for treatment with olaparib (Lynparza)). This assay is only performed at Myriad Genetic Laboratories, a single laboratory site located in Salt Lake City, UT.

In clinical studies, BRACAnalysis CDx was proven to effectively identify patients with BRCA mutations who would be candidates for Lynparza (Myriad, 2014). The FDA concurrently approved olaparib (Lynparza), a poly ADP-ribose polymerase (PARP) inhibitor that blocks enzymes involved in repairing damaged DNA. Olaparib is approved by the FDA as monotherapy in patients with deleterious or suspected deleterious germline BRCA mutated (as detected by an FDA-approved test) advanced ovarian cancer who have been treated with three or more prior lines of chemotherapy.
The FDA's approval of the companion diagnostic is based on data from the clinical study used to support approval of the new drug. Blood samples from clinical trial participants were tested to validate the test’s use for detecting BRCA mutations in this population. The agency evaluated BRACAnalysis CDx’s safety and efficacy under its premarket approval pathway used for high-risk medical devices. Myriad had been marketing this test without FDA approval as a laboratory developed test, although not specifically for use as a companion diagnostic.

On January 12, 2018, the FDA expanded the indication for BRACAnalysis CDx for use as a companion diagnostic to identify patients with HER2-negative metastatic breast cancer who have a germline BRCA mutation and are candidates for treatment with the PARP inhibitor Lynparza (olaparib) (FDA, 2018).

Robson et al. (2017) conducted a randomized, open-label, phase 3 trial in which olaparib monotherapy was compared with standard therapy in patients with a germline BRCA mutation and HER2-negative metastatic breast cancer who had received no more than two previous chemotherapy regimens for metastatic disease. Patients (n=302) were randomly assigned, in a 2:1 ratio, to receive olaparib tablets (n=205; 300 mg twice daily) or standard therapy (n=97) with single-agent chemotherapy of the physician's choice (capecitabine, eribulin, or vinorelbine in 21-day cycles). The primary end point was progression-free survival, which was assessed by blinded independent central review and was analyzed on an intention-to-treat basis. Median progression-free survival was significantly longer in the olaparib group than in the standard-therapy group (7.0 months vs. 4.2 months; P<0.001). The response rate was 59.9% in the olaparib group and 28.8% in the standard-therapy group. The rate of grade 3 or higher adverse events was 36.6% in the olaparib group and 50.5% in the standard-therapy group, and the rate of treatment discontinuation due to toxic effects was 4.9% and 7.7%, respectively. Clinical outcomes has shown median progression-free survival 2.8 months longer, and the risk of disease progression or death 42% lower with olaparib monotherapy than with standard therapy. The authors concluded that olaparib monotherapy provided a significant benefit over standard therapy among patients with HER2-negative metastatic breast cancer and a germline BRCA mutation.

**Platelet Reactivity/Function Testing (VerifyNow P2Y12 Assay)**

Clinical evidence has been controversial regarding the influence of clopidogrel on treatment platelet reactivity and ischemic outcomes. Brar et al (2011) systematically evaluated the significance of platelet reactivity on clopidogrel treatment on adverse cardiovascular events using a collaborative meta-analysis using patient-level data for the
VerifyNow P2Y12 assay (Accumetrics, San Diego, CA). MEDLINE, Scopus, and the Cochrane library databases were searched through January 2010. A database containing individual patient-level time-to-event data was generated from identified studies. The primary outcome of interest was a composite of death, myocardial infarction (MI), or stent thrombosis (STh). Secondary outcomes included the incidence of:

I. death;

II. MI; and

III. STh.

A total of 6 studies with 3,059 patients were included. In each study, clopidogrel responsiveness was assessed using the same point-of-care assay after percutaneous coronary intervention (PCI). The primary endpoint occurred more frequently in higher quartiles of P2Y(12) reaction unit (PRU) values: quartile I, 5.8 %; quartile II, 6.9 %; quartile III, 10.9 %; quartile IV, 15.8 % (p < 0.001). Taking quartile I as referent, the hazard ratios (HRs) for the primary endpoint were as follows: quartile II, HR: 1.13 (95 % CI: 0.72 to 1.78; p = 0.60); quartile III, HR: 1.82 (95 % CI: 1.20 to 2.75; p = 0.005); quartile IV, HR: 2.62 (95 % CI: 1.78 to 3.87; p < 0.001). On a continuous scale, every 10-U increase in PRU was associated with a significantly higher rate of the primary endpoint (HR: 1.04; 95 % CI: 1.03 to 1.06; p < 0.0001). According to receiver-operating characteristic (ROC) curve analysis, a PRU value of 230 appeared to best predict death, MI, or STh (p < 0.001). A PRU value greater than or equal to 230 was associated with a higher rate of the composite primary endpoint (HR: 2.10; 95 % CI: 1.62 to 2.73; p < 0.0001), as well as the individual endpoints of death (HR: 1.66; 95 % CI: 1.04 to 2.68; p = 0.04), MI (HR: 2.04; 95 % CI: 1.51 to 2.76; p < 0.001), and STh (HR: 3.11; 95 % CI: 1.50 to 6.46; p = 0.002). The authors concluded that in this collaborative meta-analysis, the level of on-treatment platelet reactivity according to the P2Y(12) assay is associated with long-term cardiovascular events after PCI, including death, MI, and STh.
Gurbel and Tantry (2011) noted that platelet-mediated thrombosis is a dreaded clinical event and is the primary cause of acute coronary syndromes (ACS) and post-PCI ischemic events. There has been a long-standing interest in the ex-vivo quantification of platelet reactivity to assess the risk of thrombosis. Early studies demonstrated platelet activation and heightened platelet reactivity in ACS and after PCI. However, a demonstration that heightened reactivity actually precipitated the ischemic event was lacking. Knowledge of platelet receptor physiology and the advent of novel inhibitors have significantly advanced the field. The P2Y12 receptor has been shown to play a pivotal role in the amplification of platelet activation by multiple agonists and its inhibition has resulted in improved clinical outcomes. The most widely used drug to block P2Y12 receptor, clopidogrel, is associated with resistance in selected patients and these patients have been shown to be at increased risk for post-PCI ischemic event occurrence in multiple studies. Importantly, a threshold of high platelet reactivity has been demonstrated, and beyond this threshold ischemic events occur precipitously. Based on the current evidence, it is rational to quantify the intensity of the ADP-P2Y12 interaction in the patient at the greatest risk for thrombosis -- the PCI patient. However, there is only evidence from small clinical trials demonstrating the clinical efficacy of changing an anti-platelet regimen based on an ex-vivo platelet function measurement. Moreover, there are numerous patients with vulnerable coronary anatomy that have not yet experienced plaque rupture; the prognostic role of a measurement of platelet reactivity in the latter group has never been studied. The authors stated that large-scale trials are ongoing that will investigate the role of personalized anti-platelet therapy in the PCI patient.

Holmes et al (2011) appraised evidence on the association of CYP2C19 genotype and clopidogrel response through systematic review and meta-analysis. PubMed and EMBASE from their inception to October 2011 were searched. Studies that reported clopidogrel metabolism, platelet reactivity or clinically relevant outcomes (cardiovascular disease [CVD] events and bleeding), and information on CYP2C19 genotype were included. These researchers extracted information on study design, genotyping, and disease outcomes and investigated sources of bias. They retrieved 32 studies of 42,016 patients reporting 3,545 CVD events, 579 STh, and 1,413 bleeding events. Six studies were randomized trials (“effect-modification” design) and the remaining 26 reported individuals exposed to clopidogrel (“treatment-only” design). In treatment-only analysis, individuals with 1 or more CYP2C19 alleles associated with lower enzyme activity had lower levels of active clopidogrel metabolites, less platelet inhibition, lower risk of bleeding (relative risk [RR], 0.84; 95 % CI: 0.75 to 0.94; absolute risk reduction of 5 to 8 events per 1,000 individuals), and higher risk of CVD events (RR, 1.18; 95 % CI: 1.09 to 1.28; absolute risk increase of 8 to 12 events per 1,000 individuals). However, there was evidence of small-study bias (Harbord test p = 0.001). When analyses were
restricted to studies with 200 or more events, the point estimate was attenuated (RR, 0.97; 95 % CI: 0.86 to 1.09). In effect-modification studies, CYP2C19 genotype was not associated with modification of the effect of clopidogrel on CVD end points or bleeding (p > 0.05 for interaction for both). Other limitations included selective outcome reporting and potential for genotype mis-classification due to problems with the * allele nomenclature for cytochrome enzymes. The authors concluded that although there was an association between the CYP2C19 genotype and clopidogrel responsiveness, overall there was no significant association of genotype with cardiovascular events.

Varenhorst et al (2011) noted that insufficient platelet inhibition is a major determinant of STh, although the etiology is multi-factorial. These investigators examined on-clopidogrel platelet reactivity in patients with previous angiographically confirmed STh, MI, and controls. Using the Swedish Coronary Angiography and Angioplasty Registry, these researchers identified patients with angiographically confirmed STh (n = 48) or MI (n = 30) while on dual anti-platelet therapy within 6 months of PCI and matched control patients (n = 78). On-clopidogrel platelet reactivity was measured with VerifyNow P2Y12 and vasodilator-stimulated phosphoprotein (VASP) phosphorylation assay. The mean PRU was higher (246.8 +/- 75.9 versus 200.0 +/- 82.7, p = 0.001) in STh patients compared with controls. The optimal cut-off for STh was 222 PRU or higher (area under the curve 0.69, p < 0.0001) in a ROC analysis. The cut-off level resulted in 70.2 % sensitivity and 67.3 % specificity. There was no significant difference in mean PRU but a higher device-reported percent inhibition (45.1 +/- 23.8 versus 32.1 +/- 23.2, p = 0.04) in patients with MI compared with controls. Results with the VASP phosphorylation assay were not related to the occurrence of STh or MI. The authors concluded that STh was associated with high on-clopidogrel platelet reactivity measured with VerifyNow (cut-off level of PRU greater than or equal to 222), but spontaneous MI in stented patients on clopidogrel treatment was not. There was, however, a substantial overlap in on-clopidogrel platelet reactivity between patients with and without on-treatment STh questioning the clinical use of platelet function testing to identify patients at high-risk for STh.

In a randomized, double-blind, active-control trial (Gauging Responsiveness with A VerifyNow assay-Impact on Thrombosis And Safety [GRAVITAS]), Price et al (2011) evaluated the effect of high-dose compared with standard-dose clopidogrel in patients with high on-treatment platelet reactivity after PCI. A total of 2,214 patients with high on-treatment reactivity 12 to 24 hours after PCI with drug-eluting stents at 83 centers in North America between July 2008 and April 2010 were included in this study. Patients received high-dose clopidogrel (600-mg initial dose, 150 mg daily thereafter) or standard-dose clopidogrel (no additional loading dose, 75 mg daily) for 6 months. The primary end point was the 6-month incidence of death from cardiovascular causes, non-
fatal MI, or STh. The key safety end point was severe or moderate bleeding according to the Global Utilization of Streptokinase and t-PA for Occluded Coronary Arteries (GUSTO) definition. A key pharmacodynamic end point was the rate of persistently high on-treatment reactivity at 30 days. At 6 months, the primary end point had occurred in 25 of 1,109 patients (2.3 %) receiving high-dose clopidogrel compared with 25 of 1,105 patients (2.3 %) receiving standard-dose clopidogrel (hazard ratio [HR], 1.01; 95 % CI: 0.58 to 1.76; p = 0.97). Severe or moderate bleeding was not increased with the high-dose regimen (15 [1.4 %] versus 25 [2.3 %], HR, 0.59; 95 % CI: 0.31 to 1.11; p = 0.10). Compared with standard-dose clopidogrel, high-dose clopidogrel provided a 22 % (95 % CI: 18 % to 26 %) absolute reduction in the rate of high on-treatment reactivity at 30 days (62 %; 95 % CI: 59 % to 65 % versus 40 %; 95 % CI: 37 % to 43 %; p < 0.001). The authors concluded that among patients with high on-treatment reactivity after PCI with drug-eluting stents, the use of high-dose clopidogrel compared with standard-dose clopidogrel did not reduce the incidence of death from cardiovascular causes, non-fatal MI, or STh.

Sharma et al (2012) noted that the substantial reduction in ischemic events provided by the dual anti-platelet regimen with aspirin and clopidogrel is well-documented in patients with ACS and patients undergoing PCI. Recently the variable response to the anti-platelet agents has received considerable attention after several "boxed warnings" on clopidogrel. This led to intense controversy on pharmacokinetic, pharmacodynamic, and pharmacogenomic issues of anti-platelet drugs, especially clopidogrel. Research use of platelet function testing has been successfully validated in identifying new anti-platelet drugs like prasugrel and ticagrelor. These platelet function assays are no longer regarded just as a laboratory phenomenon but rather as tools that have been shown to predict mortality in several clinical trials. It is believed that suboptimal response to an anti-platelet regimen (pharmacodynamic effect) may be associated with cardiovascular, cerebrovascular, and peripheral arterial events. There has been intense controversy about this variable response of anti-platelet drugs and the role of platelet function testing to guide anti-platelet therapy. While the importance of routine platelet function testing may be uncertain, it may be useful in high-risk patients such as those with diabetes mellitus, diffuse 3-vessel coronary artery disease, left main stenosis, diffuse atherosclerotic disease, and those with chronic renal failure undergoing PCI. It could also be useful in patients with suspected pharmacodynamic interaction with other drugs to assure the adequacy of platelet inhibition. The authors stated that "While we wait for definitive trials, a predictive prognostic algorithm is necessary to individualize antiplatelet therapy with P2Y12 inhibitors based on platelet function assays and genetic testing".
Yu et al (2012) confirmed the predictive cut-off values for PRU and aspirin reaction units (ARU) and evaluated the clinical impact of VerifyNow® assays. From November 2007 to October 2009, a total of 186 eligible patients were prospectively recruited. Post-treatment platelet reactivity was measured by VerifyNow® assays within 12 to 24 hours after intervention, followed by standard dual maintenance dose therapy for 1 year. All patients had scheduled clinical follow-ups at 1, 3, 6, and 12 months. The rate of low-responders to clopidogrel, aspirin, and both drugs were 41.4 %, 10.2 %, and 3.8 %, respectively. The predictive factors for low-responsiveness to clopidogrel (PRU greater than or equal to 240) were female sex, age, and non-use of cilostazol medication in uni-variate analysis and age greater than or equal to 65 years and non-use cilostazol in the multi-variate analysis. The predictors of low-responsiveness to aspirin (ARU greater than or equal to 550) were male sex and age in both uni-variate and multi-variate analyses. There was no significant difference in the clinical event rate with a cut-off value of PRU greater than or equal to 240 or ARU greater than or equal to 550 for 30 days and 1-year (p > 0.05). The authors concluded that hypo-responsiveness to anti-platelet agents (namely aspirin and clopidogrel) was identified in about 50 % of the patients. The cut-off point of PRU greater than or equal to 240 or ARU greater than or equal to 550 did not confer predictive value for 30-day or 1-year clinical event rates in patients who had undergone PCI with drug-eluting stents.

Kim et al (2012) stated that although adjunctive cilostazol to dual anti-platelet therapy can reduce the risks of clinical events after PCI, whether genetic polymorphism can influence the pharmacodynamics of this regimen has not been evaluated. In this study, a total of 127 patients treated with PCI and taking triple anti-platelet therapy (greater than or equal to 1 month) were enrolled. Platelet reactivity was assessed by conventional aggregometry and the VerifyNow P2Y12 assay. High on-treatment platelet reactivity (HPR) was defined as 5 µm ADP-induced maximal platelet reactivity (Agg(max)) greater than 46 %. CYP3A5*3, CYP2C19*2/*3 and ABCB1 3435C > T were genotyped. CYP3A5*3 and ABCB1 3435C > T variants did not affect the anti-platelet effect of triple anti-platelet therapy. For non-carriers, 1 and 2 carriers of the CYP2C19 loss-of-function (LOF) allele, Agg(max) consecutively increased after the addition of 5 µm [mean (95 % CI): 24.6 % (20.8 to 28.5 %) versus 28.7 % (25.4 to 32.0 %) versus 32.3 % (25.8 to 38.7 %), p = 0.062, respectively] and 20 µm ADP [34.2 % (29.3 to 39.0 %) versus 41.7 % (37.8 to 45.6 %) versus 44.9 % (37.9 to 51.9 %), p = 0.007, respectively]. Likewise, late platelet reactivity and PRU proportionally changed according to the number of CYP2C19 LOF alleles. High on-treatment platelet reactivities were observed in 9.2 % of subjects: 6.3 %, 7.4 % and 20.0 % with 0, 1 and 2 carriers of CYP2C19 LOF allele(s) (p = 0.099). In multi-variate analysis, carriage of 2 CYP2C19 LOF alleles was a significant predictor for the prevalence of HPR (odds ratio 5.78, 95 % CI: 1.21 to 27.78, p = 0.028). The authors concluded that among PCI-treated patients, the effect of triple anti-
platelet therapy is influenced by the CYP2C19 LOF allele. They stated that its clinical benefit needs to be validated according to the CYP2C19 metabolic phenotype in future clinical trials.

O’Connor et al (2012) noted that clopidogrel used in conjunction with aspirin has a central role in the treatment of patients with an ACS and/or undergoing PCI. The pharmacokinetic and pharmacodynamic responses to this drug are highly variable leaving up to 1/3 of patients with inadequate platelet inhibition or HPR, and subsequent increased ischemic cardiovascular events. Genetic variability in drug absorption and metabolism is a key factor responsible for the inefficient generation of the active drug metabolite. The 2-step hepatic cytochrome P450 (CYP)-dependant oxidative metabolism of the pro-drug appears to be of particular importance. Pharmacogenomic analyses have identified LOF variant alleles of CYP 2C19 and specifically the 2C19*2 allele, to be the predominant genetic mediators of the anti-platelet effect of clopidogrel. Carriers were have been shown to have lower active metabolite levels of clopidogrel, higher platelet reactivity and associated poorer outcomes. Rapid and accurate point-of-care genetic tests to identify these alleles are currently in development but several questions about the role of such testing remain such as patient selection and whether personalized treatment based on genotype has a positive impact on clinical outcome. At present, genetic testing can not be recommended in routine clinical practice due to insufficient prospective data. However, the significant body of research published to date suggests a likely role when used in combination with platelet function analysis in ACS patients undergoing stenting who have other known risk factors for recurrent ischemic events.

Mallouk et al (2012) performed a systematic review to estimate the prevalence of poor biological response to clopidogrel and investigated the factors known to modulate this. An exhaustive search was performed. A total of 171 publications were identified, providing data for 45,664 subjects. The estimated prevalence of poor biological response to clopidogrel ranged from 15.9 % to 49.5 % according to the platelet function assay employed. The assays most frequently used were light transmittance aggregometry (LTA), the vasodilator-stimulated phosphoprotein (VASP) assay and the Verifynow® assay. For all these assays, higher cut-off values were associated with a lower prevalence of poor biological response to clopidogrel. However, when choosing a fixed cut-off point for each assay, the prevalence of poor biological response to clopidogrel was highly variable suggesting that other factors could modulate poor biological response to clopidogrel. Finally, none of the studied factors could apparently explain the variability of poor biological response to clopidogrel. This meta-analysis showed that the prevalence of poor biological response depends on the assay employed, the cut-off value and on various unidentified additional factors.
A draft AHRQ report (2012) found evidence that high on-clopidogrel platelet reactivity is associated with an increased risk of adverse cardiovascular outcomes for at least some of the available assays. The report stated that the strength of evidence regarding these prognostic effects is low because of concerns regarding selective outcome reporting and the relatively small number of studies reporting clinical outcomes. The report concluded that strength of evidence regarding the use of platelet reactivity testing to guide anti-platelet treatment selection is insufficient, because studies reporting on clinical outcomes are few, have diverse designs, and included heterogeneous populations.

In summary, there is currently insufficient evidence to support the use of platelet reactivity/function testing for patients who have undergone after PCI. The incorporation of platelet reactivity/function testing into clinical practice awaits the results of ongoing clinical studies where treatment is changed based on platelet reactivity/function testing data.

**Beta adrenergic receptor genotyping (ADRB2) for treatment-resistant asthma**

ADRB2 (beta adrenergic receptor genotyping) is a genetic test used for asthma patients with poor symptom control. The target for beta2-agonist asthma medication is the B2-adrenergic receptor. The gene for the B2-adrennergic receptor is ADRB2. It is believed that a variation at one location of this gene may predict therapeutic responses to beta2-agonists. The Arg/Arg homozygous genotype at amino acid position 15 may indicate a need for a change in medication.

The textbook *Cleveland Clinic: Current Clinical Medicine* (2010) states that, in the presence of a polymorphism, the acute bronchodilator response to a β agonist, or protection from a bronchoconstrictor, may be affected. Studies indicate that in patients with Arg16Arg variant, the resulting β2-adrenergic receptor is resistant to endogenous circulating catecholamines (i.e., receptor density and integrity are preserved), with a subsequent ability to produce an acute bronchodilator response to an agonist). There are conflicting data regarding whether Arg/Arg homozygotes are prone to experience reflex morbidity with inhaled LABA, but the weight of evidence, particularly from more-recent studies, indicates that response to LABA when used in combination with ICS does not vary based on β2-adrenergic genotypes at codon 16.

In 2010, Bleecker and colleagues examined whether the response to salmeterol alone or in combination with an inhaled corticosteroid is influenced by beta- receptor polymorphisms. Subjects using only as-needed albuterol were screened and completed
two sequential open-label run-in periods (8 wk on as-needed albuterol; 8 wk on as-needed ipratropium). Five hundred forty-four subjects were randomized by Arg16Gly genotype to salmeterol alone or with fluticasone propionate for 16 weeks. Change from baseline in morning peak expiratory flow was the primary endpoint. Lung function responses were sustained over treatment and no statistically significant changes from baseline between genotypes within treatments were observed. Overall mean changes in morning peak flow for salmeterol with fluticasone propionate were 32.6 L/min (Arg/Arg vs. Gly/Gly, 95% confidence interval [CI], -6.3, 22.1), 25.9 L/min (Arg/Arg vs. Arg/Gly, 95% CI, -7.1, 21.3), and 24.9 L/min (Arg/Gly vs. Gly/Gly, 95% CI, -13.0, 14.6), and for salmeterol alone were 19.4 L/min (Arg/Arg vs. Gly/Gly, 95% CI, -1.7, 21.4), 24.6 L/min (Arg/Arg vs. Arg/Gly, 95% CI, -13.0, 10.6), and 12.4 L/min (Arg/Gly vs. Gly/Gly, 95% CI, -0.2, 22.3) for Arg/Arg, Arg/Gly, and Gly/Gly genotypes, respectively. Other measures of asthma control showed similar responses. The results showed no evidence of a pharmacogenetic effect of beta-receptor variation on salmeterol response.

Basu and colleagues (2009) investigated whether the presence of Arg16 allele of the adrenergic beta(2)-receptor agonist gene (ADRB2) predisposed to exacerbations in young asthmatic patients taking regular salmeterol. Arg/Gly status at position 16 of ADRB2 was assessed in 1182 asthmatic patients. Asthma exacerbations, use of beta-agonists and other medications over the previous 6 months, and lung function were also studied. An increased risk of exacerbations per copy of he Arg16 allele was observed in asthmatic patients, regardless of treatment regimen (odds ratio [OR], 1.30; 95% CI, 1.09-1.55; P = .003). This appeared to be largely due to exposure to beta(2)-agonists because the risk of exacerbations observed in patients with the Arg16 allele was only observed in those receiving daily inhaled long- or short-acting beta(2)-agonist treatment (OR, 1.64; 95% CI, 1.22-2.20; P = .001). In contrast, there was no genotypic risk for exacerbations in patients using inhaled beta(2)-agonists less than once a day (OR, 1.08; 95% CI, 0.85-1.36; P = .525). The Arg16 genotype-associated risk for exacerbations was significantly different in those exposed to beta(2)-agonists daily versus those that were not (test for interaction, P = .022). The authors concluded that Arg16 genotype of ADRB2 was associated with exacerbations in asthmatic children and young adults exposed daily to beta(2)-agonists, regardless of whether the exposure is to albuterol or long-acting agonists, such as salmeterol.

In a 2008 article, Martin and associates evaluated the influence of single nucleotide polymorphisms in the beta(2)-adrenoceptor gene, on the response to inhaled beta(2)-agonists in children with acute asthma. One hundred and forty-eight children with acute asthma were recruited and genotyped for beta(2)Arg16Gly and beta(2)Gln27Glu. For Gln27Glu, individuals Gln27Gln took longest to stretch out to 1, 2 and 4 hourly beta(2)-agonists, followed by heterozygotes who were intermediate and Glu27Glu who
responded most rapidly (1 hourly: 2.6 hr vs. 2.0 vs. 1.4, p = 0.02; 2 hourly: 10.6 hr vs. 10.7 vs. 6.8, p = 0.07; 4 hourly: 29.8 hr vs. 28.5 vs. 24.3, p = 0.30). The authors reported that the ability to prospectively identify children who respond less effectively to beta (2)-agonists during an acute asthma attack has the potential to allow the generation of genotype-specific treatment pathways.

In 2008, Giubergia et al assessed the frequency of beta2-adrenergic receptor (beta2-AR) polymorphisms in asthmatic children from Argentina, and evaluated their influence on bronchodilator desensitization to albuterol over a 4-week treatment. beta2-AR genotypes were determined in 117 children with asthma and 101 of them were under 4 weeks treatment with albuterol. Spirometric changes in FEV(1) were recorded at the beginning (day 1) and at the end of the study (day 30) and compared to genotypes at position 16 and 27 of the receptor. The frequency of the polymorphisms was calculated in all population. The presence of glutamine at position 27 (Gln27) was significantly more frequent in this Argentinean study population than in other Caucasian populations. The homozygosity for Gln27 polymorphism was associated to a desensitization of the receptor with a decline in the bronchodilator response to albuterol after chronic use.

In 2007, Bleeker et al investigated whether beta2-adrenergic receptor (ADRB2) polymorphisms affect response to longacting beta2-agonists in combination with inhaled corticosteroids. Asthmatics were stratified by ADRB2 genotype in two studies to assess the effects of inhaled corticosteroids plus longacting beta2-agonists on asthma exacerbations. In study 1 (double-blind), 2250 asthmatics were randomly assigned to budesonide plus formoterol maintenance and reliever therapy, fixed-dose budesonide plus formoterol, or fixed-dose fluticasone plus salmeterol for 6 months. Study 2 (open-label) consisted of 405 asthmatics and compared an adjustable regimen of budesonide plus formoterol with fixed-dose budesonide plus formoterol and fixed-dose fluticasone plus salmeterol for 7 months. The relation between ADRB2 polymorphism, severe asthma exacerbations, and other asthma outcomes was analysed. Primary endpoints for studies 1 and 2 were severe asthma exacerbation and asthma control as assessed by measures of exacerbations, respectively. In study 1, Gly16Arg genotype had no effect on the percentage of participants with severe exacerbations across all treatment groups (99 [12%] of 833 Gly/Gly, 110 [11%] of 1028 Gly/Arg, and 32 [9%] of 361 Arg/Arg participants). Secondary endpoints, including forced expiratory volume in 1 s, peak expiratory flow, use of as-needed medication, and number of nights with awakenings were similar between genotype groups. No relation was recorded between ADRB2 haplotype and primary and secondary endpoints. In study 2, the frequency of asthma exacerbations (15 [9%] of 168 Gly/Gly, 13 [8%] of 169 Gly/Arg, and 6 [9%] of 67 Arg/Arg participants) and other study endpoints were closely similar for all ADRB2 genotypes.
Hawkins and colleagues (2006) sought to identify ADRbeta2 polymorphisms and haplotype structure in white and African American subjects and to test for genotype and haplotype association with asthma phenotypes. A 5.3-kb region of ADRbeta2 was resequenced in 669 individuals from 429 whites and 240 African Americans. A total of 12 polymorphisms, representing an optimal haplotype tagging set, were genotyped in whites (338 patients and 326 control subjects) and African Americans (222 patients and 299 control subjects). A total of 49 polymorphisms were identified, 21 of which are novel; 31 polymorphisms (frequency > 0.03) were used to identify 24 haplotypes (frequency > 0.01) and assess linkage disequilibrium. Association with ratio (FEV1/FVC)² for single-nucleotide polymorphism +79 (p < 0.05) was observed in African Americans. Significant haplotype association for (FEV1/FVC)² was also observed in African Americans. The authors concluded, “these data suggest that the length of a poly-C repeat (+1269) in the 3’ untranslated region of ADRbeta2 may influence lung function, and may be important in delineating variation in beta-agonist responses, especially in African Americans.”

Litonjua (2006) writes the gene that encodes the beta2-adrenergic receptor (ADRB2) is one of the most studied candidate genes in asthma. Candidate gene association studies of ADRB2 and asthma have been dominated by analyses of the two common non-synonymous coding single nucleotide polymorphisms, Arg16Gly and Glu27Gly. Published studies have yielded inconsistent results. Three recent meta-analyses on the effects of these two polymorphisms have found no associations with asthma, although there were suggestions of associations with other asthma-related phenotypes, such as nocturnal asthma and asthma severity. Other recent studies have investigated other single nucleotide polymorphisms in this gene (i.e. single nucleotide polymorphisms in the promoter region and other single nucleotide polymorphisms in the coding region). These analyses have investigated the association between these single nucleotide polymorphisms (and haplotypes of these polymorphisms) and asthma-related phenotypes such as lung function, airways hyperresponsiveness, and response to a bronchodilator, and have suggested that certain regions of the gene may be associated with different phenotypes. Results from these studies, however, have also been inconsistent. Polymorphisms of ADRB2 are not major risk factors for the development of asthma. These polymorphisms are likely to be important, however, in determining drug response. Future studies need to fully characterize all of the variation in the gene and perform comprehensive association studies. Finally, interactions between ADRB2 and other genes in the beta-agonist pathway are an important and active area of research that will shed more light on inter-individual differences in drug response.

with inhaled beta2-agonists and corticosteroids. ADRB2 haplotype was determined in 176 patients with asthma, of whom 161 harbored the six most common combinations. There were no significant differences in bronchodilator response (% improvement in FEV(1)) with respect to any of the major ADRB2 haplotypes or genotypes. The authors concluded, “genetic variation of the ADRB2 does not influence the immediate response to inhaled beta2-agonist. The confounding effect of tolerance resulting from regular beta2-agonist use must be controlled when assessing the pharmacogenetic influences on clinical outcomes with beta2-agonists.”

According to Johnson (2006), the presence of a particular form of the B2-receptor, which might influence clinical efficacy of regular B2-agonists, is of increasing interest in predicting good and poor responders to therapy.

**MGMT Gene Methylation Assay for Gliomas**

Glioblastomas are among the most aggressive of all known human tumors. The median survival times remain in the 12 to 15-month range despite aggressive surgery, radiation, and chemotherapy. Through molecular and genetic profiling efforts, underlying mechanisms of resistance to these therapies are becoming better understood. Resistance to alkylating agents via direct DNA repair by O(6)-methylguanine methyltransferase (MGMT) has been investigated as a barrier to successful treatment. Assessment of MGMT status could help identify gliomas patients more likely to respond to chemotherapy or to benefit from MGMT depletion strategies. Strategies to overcome MGMT-mediated chemoresistance are being actively investigated.

Current guidelines from the National Comprehensive Cancer Network (NCCN, 2012) include recommendations for the use of the MGMT gene methylation assay in assessing likelihood of response to temozolomide in glioblastoma. The guidelines recommend use of temozolomide if methylguanine methyl-transferase [MGMT] promoter methylation is positive in persons with glioblastoma.

In a review, Hegi et al (2008) stated that one strategy to overcome MGMT-mediated chemoresistance includes treatment with nontoxic pseudo-substrate inhibitors of MGMT, such as O(6)-benzylguanine, or RNA interference-mediated gene silencing of MGMT. However, the author reported that systemic application of MGMT inhibitors is limited by an increase in hematologic toxicity. Another strategy, the author explained, is to deplete MGMT activity in tumor tissue using a dose-dense temozolomide schedule. The author stated that these alternative schedules are well tolerated; however,
it remains unclear whether they are more effective than the standard dosing regimen or whether they effectively deplete MGMT activity in tumor tissue. The author noted that not all patients with glioblastoma having MGMT promoter methylation respond to alkylating agents, and even those who respond will inevitably experience relapse.

Data from a retrospective study reported by Cancer Care Ontario (2006) suggested that newly diagnosed malignant glioma patients who had undergone surgery and external beam radiotherapy had a greater benefit from temozolomide treatment if their tumors had MGMT promoter methylation versus patients with unmethylated MGMT tumors. However, it should be noted that these studies were performed retrospectively and prospective validation is required before MGMT methylation can be used for clinical stratification purposes.

Idbaih et al (2007) reviewed recent studies on molecular markers including MGMT in the treatment of glioma and found evidence that MGMT inactivation is a prognostic marker and predictor of chemosensitivity in gliomas. The author stated, "Although such markers remain to be formally validated by ongoing and planned prospective trials, it is likely that they will soon become essential for optimizing treatment decisions."

**Millennium PGT**

According to the Millennium Laboratories, Millennium PGT - Pharmacogenetic Testing is saliva-based testing designed to detect genetic variations in enzymes associated with the metabolism of medications commonly prescribed to patients suffering from chronic pain and pain-related effects. Millennium PGT is designed to help clinicians more effectively personalize treatment by identifying patients who may benefit from modifying the drug selection or dosing of certain prescribed opioids, including codeine, hydrocodone, oxycodone, tramadol, and methadone; and benzodiazepines; tricyclic antidepressants (TCAs); selective serotonin reuptake inhibitors (SSRIs); and serotonin norepinephrine reuptake inhibitors (SNRIs). There is a lack of peer-reviewed publications on the Millennium PGT.

Vuilleumier et al (2012) stated that translating pharmacogenetics to clinical practice has been particularly challenging in the context of pain, due to the complexity of this multi-faceted phenotype and the overall subjective nature of pain perception and response to analgesia. Overall, numerous genes involved with the pharmacokinetics and dynamics of opioids response are candidate genes in the context of opioid analgesia. The clinical relevance of CYP2D6 genotyping to predict analgesic outcomes is still relatively unknown; the 2 extremes in CYP2D6 genotype (ultra-rapid and poor metabolism) seem
to predict pain response and/or adverse effects. Overall, the level of evidence linking genetic variability (CYP2D6 and CYP3A4) to oxycodone response and phenotype (altered biotransformation of oxycodone into oxymorphone and overall clearance of oxycodone and oxymorphone) is strong; however, there has been no randomized clinical trial on the benefits of genetic testing prior to oxycodone therapy. On the other hand, predicting the analgesic response to morphine based on pharmacogenetic testing is more complex; though there was hope that simple genetic testing would allow tailoring morphine doses to provide optimal analgesia, this is unlikely to occur. A variety of polymorphisms clearly influence pain perception and behavior in response to pain. However, the response to analgesics also differs depending on the pain modality and the potential for repeated noxious stimuli, the opioid prescribed, and even its route of administration.

Svetlik et al (2013) reviewed the impact of genetic variability of drug metabolizing enzymes, transporters, receptors, and pathways involved in chronic pain perception on the efficacy and safety of analgesics and other drugs used for chronic pain treatment. Several candidate genes have been identified in the literature, while there is usually only limited clinical evidence substantiating for the penetration of the testing for these candidate biomarkers into the clinical practice. Moreover, the authors noted that the pain-perception regulation and modulation are still not fully understood, and thus more complex knowledge of genetic and epigenetic background for analgesia will be needed prior to the clinical use of the candidate genetic biomarker.

Thiopurine S-Methyltransferase (TPMT)

Thiopurine s-methyltransferase (TPMT) is an enzyme that is involved in the metabolism of medications called thiopurines that are used in the treatment of inflammatory bowel disease (IBD). Genotyping tests (eg, PRO-PredictRx) and phenotyping tests (eg, PRO-Predict EnzAct) for TPMT enzyme activity can be used to help make treatment decisions involving thiopurine medications such as azathioprine and 6-mercaptopurine in IBD.

Coenen et al (2015) performed a prospective study to determine whether genotype analysis of thiopurine S-methyltransferase (TPMT) before thiopurine treatment, and dose selection based on the results, affected the outcomes of patients with inflammatory bowel disease (IBD). In a study performed at 30 Dutch hospitals, patients were assigned randomly to groups that received standard treatment (control) or pre-treatment screening (intervention) for 3 common variants of TPMT (TPMT*2, TPMT*3A, and TPMT*3C). Patients in the intervention group found to be heterozygous carriers of a variant received 50 % of the standard dose of thiopurine (azathioprine or 6-
mercaptopurine), and patients homozygous for a variant received 0 % to 10 % of the standard dose. These researchers compared, in an intention-to-treat analysis, outcomes of the intervention (n = 405) and control groups (n = 378) after 20 weeks of treatment. Primary outcomes were the occurrence of hematologic adverse drug reactions (ADRs) (leukocyte count of less than 3.0*10(9)/L or reduced platelet count of less than 100*10(9)/L) and disease activity (based on the Harvey-Bradshaw Index for Crohn’s disease [n = 356] or the partial Mayo score for ulcerative colitis [n = 253]). Similar proportions of patients in the intervention and control groups developed a hematologic ADR (7.4 % versus 7.9 %; RR, 0.93; 95 % CI: 0.57 to 1.52) in the 20 weeks of follow-up evaluation; the groups also had similar mean levels of disease activity (p = 0.18 for Crohn’s disease and p = 0.14 for ulcerative colitis). However, a significantly smaller proportion of carriers of the TPMT variants in the intervention group (2.6 %) developed hematologic ADRs compared with patients in the control group (22.9 %) (RR, 0.11; 95 % CI: 0.01 to 0.85). The authors concluded that screening for variants in TPMT did not reduce the proportions of patients with hematologic ADRs during thiopurine treatment for IBD. However, there was a 10-fold reduction in hematologic ADRs among variant carriers who were identified and received a dose reduction, compared with variant carriers who did not, without differences in treatment efficacy.

Plumpton and colleagues (2016) stated that pharmacogenetics offers the potential to improve health outcomes by identifying individuals who are at greater risk of harm from certain medicines. Routine adoption of pharmacogenetic tests requires evidence of their cost-effectiveness. These researchers conducted a systematic literature review of economic evaluations of pharmacogenetic tests aimed to reduce the incidence of ADRs. Literature was searched using Embase, Medline and the NHS Economic Evaluation Database with search terms relating to pharmacogenetic testing, adverse drug reactions, economic evaluations and pharmaceuticals. Titles were screened independently by 2 reviewers. Articles deemed to meet the inclusion criteria were screened independently on abstract, and full texts reviewed. These investigators identified 852 articles, of which 47 met the inclusion criteria.

There was evidence supporting the cost-effectiveness of testing for the following:

- HLA-B*57:01 (prior to abacavir)
- HLA-B*15:02 and HLA-A*31:01 (prior to carbamazepine)
- HLA-B*58:01 (prior to allopurinol)
- CYP2C19 (prior to clopidogrel treatment)

Economic evidence was inconclusive with respect to the following:
• TPMT (prior to 6-mercaptopurine, azathioprine and cisplatin therapy)
• CYP2C9 and VKORC1 (to inform genotype-guided dosing of coumarin derivatives)
• MTHFR (prior to methotrexate treatment) and factor V Leiden testing (prior to oral contraception)

Testing for A1555G is not cost-effective before prescribing aminoglycosides.

The authors concluded that systematic review identified robust evidence of the cost-effectiveness of genotyping prior to treatment with a number of common drugs. However, further analyses and (or) availability of robust clinical evidence is necessary to make recommendations for others.

**PROOVE Narcotic Risk Test**

The PROOVE narcotic risk test is comprised of 12 panels designed to predict risk of narcotic addiction. These 12 genetic assessment tests include seratonin 2A receptor, seratonin transporter, catechol-o-methyl transferase, dopamine D2 receptor dopamine D1 receptor, dopamine D4 receptor, dopamine transporter, dopamine beta hydroxylase, methylene tetrahydrofolate reductase, kappa opiod receptor, gamma-aminobutyric acid, and mu opiod receptor. The clinical utility of these tests used prior to onset of narcotic pain relief has not been established in the peer-reviewed literature.

**Aegis Drug-Drug Interaction Test**

Aegis Drug-Drug Interaction Test is urinary test that is used for drug-monitoring (120 or more drugs and substances) by means of tandem mass spectrometry with liquid chromatography. It provides a qualitative report of presence (including quantitative levels, when detected) or absence of each drug or substance with description and severity of potential interactions, with identified substances.

Chen and colleagues (2016) noted that amino acids (AAs), neurotransmitters, purines, and pyrimidines are bioactive molecules that play fundamental roles in maintaining various physiological functions. Their metabolism is closely related to the health, growth, development, reproduction, and homeostasis of organisms. Most recently, comprehensive measurements of these metabolites have shown their potential as innovative approaches in disease surveillance or drug intervention. However, simultaneous measurement of these metabolites presents great difficulties. These researchers reported a novel quantitative method that uses hydrophilic interaction ultra-high-performance liquid chromatography-tandem mass spectrometry (HILIC-UPLC-
MS/MS), which is highly selective, high throughput, and exhibits better chromatographic behavior than existing methods. The developed method enabled the rapid quantification of 37 metabolites, spanning AAs, neurotransmitters, purines, and pyrimidines pathways, within 6.5 minutes. The compounds were separated on an ACQUITY UPLC BEH amide column. Serum and brain homogenate were extracted by protein precipitation. The intra- and inter-day precision of all of the analytes was less than 11.34 %, and the accuracy was between -11.74 and 11.51 % for all quality control (QC) levels. The extraction recoveries of serum ranged from 84.58 % to 116.43 % and those of brain samples from 80.80 % to 119.39 %, while the relative standard deviation (RSD) was 14.61 % or less for all recoveries. This method was used to successfully characterize alterations in the rat brain and, in particular, their dynamics in serum. The following study was performed to simultaneously test global changes of these metabolites in a serotonin antagonist p-chlorophenylalanine (PCPA)-induced anxiety and insomnia rat model to understand the effect and mechanism of PCPA. Taken together, these results showed that the method is able to simultaneously monitor a large panel of metabolites and that this protocol may represent a metabolomic method to diagnose toxicological and pathophysiological states.

**OneOme RightMed Pharmacogenomic Test**

The OneOme RightMed Pharmacogenomic Test ascertains how individuals’ genes may affect their response to medications. The test analyzes a patient’s genetic information and provides a clinical report to aid physicians make more precise prescription decisions. The OneOme RightMed Pharmacogenomic Test includes 22 genes (CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP3A5, COMT, DPYD, DRD2, F2, F5, GRIK4, HTR2A, HTR2C, IL28B (IFNL3), NUDT15, OPRM1, SLCO1B1, TPMT, UGT1A1, and VKORC1) and entails more than 340 medications and over 20 conditions (e.g., allergies, benign prostatic hyperplasia, cancer, diabetes, gastro-esophageal reflux disease, hypertension, migraine, pain, rheumatoid arthritis, seizure, and sleep disorders). The genes tested in the OneOme RightMed Pharmacogenomic Test might affect other drugs that are not included in the Test’s pharmacogenomic database that covers approximately 1,000 of the most commonly used medications, including over 340 that are part of the RightMed Pharmacogenomic Test. The absence of a drug in the OneOme report does not imply that there is no interaction between the drug and the genes analyzed in this test. Information included in the OneOme report is based on scientific literature and does not take into account other genetic variants as well as environmental or social factors that may affect a patient’s response. Other factors not included in the report include, but are not limited to, environmental factors (e.g., smoking), health factors (e.g., diet), social and familial factors, various medical conditions and drug-drug interactions.
Hafner and colleagues (2016) stated that polymorphisms in genes encoding drug targets, drug transport proteins, or drug metabolizing enzymes may be responsible, among other factors, for the observed variation in individuals' responses to medications. The field of pharmacogenetics aims to identify patients at higher genetically-determined risk of adverse effects (AEs) or poor response to medication. This information would allow for modification of dosage or substitution with alternative therapy. However, there is a lack of awareness of pharmacogenetic clinical practice guidelines. These researchers performed a systematic review using the Medline and PharmGKB databases, which focused on published guidelines for dosage modification or selection of drugs based on germline mutations in genes with pharmacokinetic or pharmacodynamic impact. Prescribing information from the European Medicines Agency, the German Federal Institute for Drugs and Medical Devices, and the FDA was also screened for pharmacogenetic guidance. The literature review revealed 20 guideline publications elucidating 43 drugs with recommendations for genotype-guided prescribing. Moreover, drug labels for 37 drugs also contained genotype-guided prescribing recommendations, some of which were linked to optional or obligatory pre-therapeutic pharmacogenetic testing. The authors concluded that existing guidelines for genotype-based drug prescribing are rarely derived from prospective, controlled trials; thus, their level of evidence is usually low. Even with low-quality evidence, strong recommendations can be made in favor of pharmacogenetic modification of prescription, such as for abacavir and the HLA-B genotype, if there is a large and certain difference between the benefits and harms. For other drug-gene pairs, such as vitamin K antagonists and CYP2C9/VKORC1, the net benefit from the pharmacogenetic-based dosing strategy is small and matter of debate. They stated that because pharmacogenetics is playing a growing role in drug development and pre-prescription genotyping will become more widespread, specific pharmacogenetic guidance for treating physicians will become increasingly important.

Berm and associates (2016) noted that as a consequence of extended application of pharmacogenetic and pharmacogenomic screening (PGx) tests it is important to examine if they provide good value for money. This review provided an update of the literature. These investigators performed a literature search in PubMed and papers published between August 2010 and September 2014, examining the cost-effectiveness of PGx screening tests. Papers from 2000 until July 2010 were included via 2 previous systematic reviews. Studies' overall quality was assessed with the Quality of Health Economic Studies (QHES) instrument. These researchers found 38 studies, which combined with the previous 42 studies resulted in a total of 80 included studies. An average QHES score of 76 was found. Since 2010, more studies were funded by pharmaceutical companies. Most recent studies performed cost-utility analysis, univariate and probabilistic sensitivity analyses, and discussed limitations of their
economic evaluations. Most studies indicated favorable cost-effectiveness. Majority of evaluations did not provide information regarding the intrinsic value of the PGx test. There were considerable differences in the costs for PGx testing. Reporting of the direction and magnitude of bias on the cost-effectiveness estimates as well as motivation for the chosen economic model and perspective were frequently missing. The authors concluded that application of PGx tests was mostly found to be a cost-effective or cost-saving strategy, although some studies concluded otherwise which underlined the importance of future studies assessing the cost-effectiveness of PGx tests. These investigators found that only the minority of recent pharmacoeconomic evaluations assessed the intrinsic value of the PGx tests. New compounds that are not affected by genetics, are emerging as cost-effective alternatives for pharmacogenetic testing strategies. Over the past 10 years, there was an increase in the number of studies and in the reporting of quality associated characteristics. Due to rapid development in analytical techniques, reporting of analytical and clinical validity of the assessed PGx test is recommended for future evaluations. Furthermore, they stated that robust clinical evidence regarding PGx tests’ effectiveness is needed.

This study had 2 main drawbacks:

1. the authors did not include studies from other databases than PubMed or grey literature and they only included English written studies. Thus, some studies might have been missed. In general, studies that are not indexed in Medline or written in English do not have a large impact on reviews’ outcomes. Nevertheless, these studies are frequently of lower quality, and therefore the average quality of the studies included in this review might have been slightly over-estimated. However, based on the comparison with other reviews the authors found a slightly lower average quality score, and

2. publication bias can always influence the findings of a review; thus, cost-effectiveness of PGx tests could be over-estimated.

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the comparison with other reviews the authors found a slightly lower average quality score, and

2. publication bias can always influence the findings of a review; thus, cost-effectiveness of PGx tests could be over-estimated.

Hinderer and co-workers (2017) noted that pharmacogenomic clinical decision support systems (CDSS) have the potential to help overcome some of the barriers for translating pharmacogenomic knowledge into clinical routine. Before developing a prototype it is crucial for developers to know which pharmacogenomic CDSS features and user-system interactions have yet been developed, implemented and tested in previous pharmacogenomic CDSS efforts and if they have been successfully applied. These researchers addressed this issue by providing an overview of the designs of user-system interactions of recently developed pharmacogenomic CDSS. They searched PubMed for pharmacogenomic CDSS published between January 1, 2012 and November 15, 2016; 32 out of 118 identified articles were summarized and included in the final analysis. These investigators then compared the designs of user-system interactions of the 20 pharmacogenomic CDSS that they had identified. Alerts were the most widespread tools for physician-system interactions, but need to be implemented carefully to prevent alert fatigue and avoid liabilities. Pharmacogenomic test results and override reasons stored in the local electronic health record might help communicate pharmacogenomic information to other internal care providers. Integrating patients into user-system interactions through patient letters and online portals might be crucial for transferring pharmacogenomic data to external health care providers. Inbox messages informed physicians about new pharmacogenomic test results and enabled them to request pharmacogenomic consultations. Search engines enabled physicians to compare medical therapeutic options based on a patient’s genotype. The authors concluded that within the last 5 years, several pharmacogenomic CDSS have been developed. However, most of the included articles were solely describing prototypes of pharmacogenomic CDSS rather than evaluating them. They stated that further evaluation efforts are needed to support the development of prototypes; in the future, pharmacogenomic CDSS will likely include prediction models to identify patients who are suitable for preemptive genotyping.

Verbelen and colleagues (2017) stated that PGx has the potential to personalize pharmaceutical treatments. Many relevant gene-drug associations have been discovered, but PGx-guided treatment needs to be cost-effective as well as clinically beneficial to be incorporated into standard health-care. These investigators reviewed economic evaluations for PGx associations listed in the FDA Table of Pharmacogenomic Biomarkers in Drug Labeling. They determined the proportion of evaluations that found PGx-guided treatment to be cost-effective or dominant over the alternative strategies,
and estimated the impact on this proportion of removing the cost of genetic testing. Of the 137 PGx associations in the FDA Table, 44 economic evaluations, relating to 10 drugs, were identified. Of these evaluations, 57% drew conclusions in favor of PGx testing, of which 30% were cost-effective and 27% were dominant (cost-saving). If genetic information was freely available, 75% of economic evaluations would support PGx-guided treatment, of which 25% would be cost-effective and 50% would be dominant. Thus, PGx-guided treatment can be a cost-effective and even a cost-saving strategy. The authors concluded that having genetic information readily available in the clinical health record is a realistic future prospect, and would make more genetic tests economically worthwhile.

Peterson and associates (2017) performed an evidence review of the effectiveness, harms, and cost-effectiveness of pharmacogenomics-guided anti-depressant treatment for major depressive disorder (MDD). They searched Medline, the Cochrane Central Registry of Controlled Trials, and PsycINFO through February 2017. They used pre-specified criteria to select studies, abstract data, and rate internal validity and strength of the evidence (PROSPERO number CRD42016036358). These researchers included 2 RCTs, 5 controlled cohort studies, and 6 modeling studies of mostly women in their mid-40s with few co-morbidities. CNSDose (ABCB1, ABCC1, CYP2C19, CYP2D6, UGT1A1) is the only pharmacogenomics test that significantly improved remission (1 additional remitting patient in 12 weeks per 3 genotyped, 95% CI: 1.7 to 3.5) and reduced intolerability in an RCT. ABCB1 genotyping leads to 1 additional remitting patient in 5 weeks per 3 genotyped (95% CI: 3 to 20), but tolerability was not reported. In an RCT, GeneSight (CYP2D6, CYP19, CYP1A2, SLC6A4, HTR2A) did not statistically significantly improve remission, and evidence is inconclusive about its tolerability. Evidence is generally low strength because RCTs were few and under-powered. Cost-effectiveness is unclear due to lack of directly observed cost-effectiveness outcomes. These researchers found no studies that evaluated whether pharmacogenomics shortened time to optimal treatment, whether improvements were due to switches to genetically congruent medication, or whether effectiveness varies based on test and patient characteristics. The authors concluded that certain pharmacogenomics tools showed promise of improving short-term remission rates in women in their mid-40s with few co-morbidities; however, important evidence limitations precluded recommending their widespread use and indicated a need for further research.

Rosenblat and associates (2017) stated that pharmacogenomics has shown promise for predicting anti-depressant response and tolerability in the treatment of MDD. In theory, pharmacogenomics can improve clinical outcomes by guiding anti-depressant selection and dosing. In a systematic review, these investigators determined the impact of pharmacogenomic testing on clinical outcomes in MDD, and evaluated its cost-
effectiveness. The Medline/PubMed and Google Scholar databases were systematically searched for relevant articles published prior to October 2015. Search terms included various combinations of the following: major depressive disorder (MDD), depression, mental illness, mood disorder, antidepressant, response, remission, outcome, pharmacogenetic, pharmacogenomics, pharmacodynamics, pharmacokinetic, genetic testing, genome wide association study (GWAS), CYP450, personalized medicine, cost-effectiveness, and pharmacoeconomics. Of the 66 records identified from the initial search, relevant clinical studies, written in English, assessing the cost-effectiveness and/or efficacy of pharmacogenomic testing for MDD were included. Each publication was critically examined for relevant data. A total of 2 non-randomized, open-label, 8-week, prospective studies reported overall greater improvement in depressive symptom severity in the group of MDD subjects receiving psychiatric care guided by results of combinatorial pharmacogenomic testing (GeneSight) when compared to the unguided group. One industry-sponsored, randomized, double-blind, 10-week prospective study reported a trend for improved outcomes for the GeneSight-guided group; however, the trend did not reach statistical significance. Another industry-sponsored, randomized, double-blind, 12-week prospective study reported a 2.5-fold increase in remission rates in the CNSDose-guided group (p < 0.0001). One naturalistic, un-blinded, industry-sponsored study showed clinical improvement when pharmacogenomics testing guided prescribing; however, this study lacked a control group. A single cost-effectiveness study concluded that single-gene testing was not cost-effective. Conversely, a separate study reported that combinatorial pharmacogenomic testing is cost-effective. The authors concluded that a limited number of studies have shown promise for the clinical utility of pharmacogenomic testing; however, cost-effectiveness of pharmacogenomics, as well as demonstration of improved health outcomes, is not yet supported with replicated evidence.

It is also interesting to note that in a systematic review on “Pharmacogenomic testing for psychotropic medication selection”, Health Quality Ontario (2017) reported that there is uncertainty about the use of GeneSight Psychotropic pharmacogenomic genetic panel to guide medication selection.

Singh et al (2015) stated major depressive disorder (MDD) is projected to be a leading cause of disability globally by 2030. Only a minority of patients remit with antidepressants. If assay of polymorphisms influencing central nervous system (CNS) bioavailability could guide prescribers to more effectively dose patients, remission rates may improve and the burden of disease from MDD reduce. Hepatic and blood brain barrier (BBB) polymorphisms appear to influence antidepressant CNS bioavailability. A 12-week prospective double blind randomized genetically guided versus unguided trial of antidepressant dosing in Caucasian adults with MDD (n=148) was conducted.
Subjects receiving genetically guided prescribing had a 2.52-fold greater chance of remission (95% confidence interval [CI]=1.71-3.73, z=4.66, p<0.0001). The number needed to genotype (NNG) was 3 (95% CI=1.7-3.5) to produce an additional remission. The authors concluded that these data suggest that a pharmacogenetic dosing report (CNSDose®) improves antidepressant efficacy. The effect size was sufficient that translation to clinical care may arise if results are independently replicated.

Bouseman et al (2017) stated pharmacogenetic-based dosing support tools have been developed to personalize antidepressant-prescribing practice. However, the clinical validity of these tools has not been adequately tested, particularly for specific antidepressants. The authors examined the concordance between the actual dose and a polygene pharmacogenetic predicted dose of desvenlafaxine needed to achieve symptom remission. A 10-week, open-label, prospective trial of desvenlafaxine among Caucasian adults with major depressive disorder (n=119) was conducted. Dose was clinically adjusted and at the completion of the trial, the clinical dose needed to achieve remission was compared with the predicted dose needed to achieve remission. Among remitters (n=95), there was a strong concordance (Kendall’s τ-b=0.84, P=0.0001; Cohen’s κ=0.82, P=0.0001) between the actual and the predicted dose need to achieve symptom remission, showing high sensitivity (≥85%), specificity (≥86%), and accuracy (≥89%) of the tool. The authors concluded that findings provide initial evidence for the clinical validity of a polygene pharmacogenetic-based tool for desvenlafaxine dosing.

CYP2C19 Polymorphisms Testing for Individuals on Fluoxetine

Luk et al (2014) stated that diazepam is often used as an adjuvant to pain therapy. Cytochrome P450 (CYP) 3A4 and 2C19 metabolize diazepam into the active metabolites: nordiazepam, temazepam and oxazepam. Owing to diazepam’s side-effect profile, mortality risk and potential for drug-drug interactions with CYP 3A4 and/or CYP 2C19 inhibitors, urine drug testing (UDT) could be a helpful monitoring tool. This was a retrospective data analysis that evaluated urine specimens from pain management practices for the distribution of diazepam metabolites with and without CYP 3A4 and 2C19 inhibitors. Inter-subject nordiazepam, temazepam and oxazepam geometric mean fractions were 0.16, 0.34 and 0.47, respectively. Intra-subject geometric mean fractions were 0.157, 0.311 and 0.494, respectively. Sex, but not age or urinary pH, had an effect on metabolite fractions. Methadone significantly increased temazepam and oxazepam urinary fractions via CYP3A4 inhibition, whereas fluoxetine and esomeprazole increased nordiazepam fractions via CYP2C19 inhibition. The authors concluded that although more studies are needed, these results suggested the viability of UDT for increased monitoring for therapy and possible drug-drug interactions.
Dinger et al (2016) noted that in-vitro cytochrome P450 (CYP) inhibition assays are common approaches for testing the inhibition potential of drugs for predicting potential interactions. In contrast to marketed medicaments, drugs of abuse, particularly the so-called novel psychoactive substances, were not tested before distribution and consumption. Therefore, the inhibition potential of methylenedioxy-derived designer drugs (MDD) of different drug classes such as aminoindanes, amphetamines, benzofurans, cathinones, piperazines, pyrrolidinophenones, and tryptamines should be elucidated. The FDA-preferred test substrates, split in 2o cocktails, were incubated with pooled human liver microsomes and analyzed after protein precipitation using LC-high-resolution-MS/MS. IC50 values were determined of MDD showing more than 50 % inhibition in the prescreening. Values were calculated by plotting the relative metabolite concentration formed over the logarithm of the inhibitor concentration. All MDD showed inhibition against CYP2D6 activity and most of them in the range of the clinically relevant CYP2D6 inhibitors quinidine and fluoxetine. In addition, the beta-keto compounds showed inhibition of the activity of CYP2B6, 5,6-MD-DALT of CYP1A2 and CYP3A, and MDAI of CYP2A6, all in the range of clinically relevant inhibitors. The authors concluded that all MDD showed inhibition of the activity of CYP2D6, 6 of CYP1A2, 3 of CYP2A6, 13 of CYP2B6, 2 of CYP2C9, 6 of CYP2C19, 1 of CYP2E1, and 6 of CYP3A. They stated that these findings showed that the CYP inhibition by MDD might be clinically relevant, but further studies are needed for final conclusions.

Bykov et al (2017) noted that clopidogrel is a pro-drug that requires activation by the cytochrome P450 (CYP) enzyme system. Patients receiving clopidogrel are often treated with SSRIs for co-existing depression. SSRIs that inhibit the CYP2C19 enzyme have the potential to reduce the effectiveness of clopidogrel. Using 5 US databases (1998 to 2013), these investigators conducted a cohort study of adults who initiated clopidogrel while being treated with either an SSRI that inhibits CYP2C19 (fluoxetine and fluvoxamine) or a non-inhibiting SSRI. Patients were matched by propensity score and followed for as long as they were exposed to both clopidogrel and the index SSRI group (primary analysis) or for 180 days after clopidogrel initiation (sensitivity analysis). Outcomes included a composite ischemic event (myocardial infarction, ischemic stroke, or a re-vascularization procedure) and a composite major bleeding event (gastro-intestinal bleed or hemorrhagic stroke). The final propensity score-matched cohort comprised 9,281 clopidogrel initiators on CYP2C19-inhibiting SSRIs and 44,278 clopidogrel initiators on a non-inhibiting SSRIs. Compared with those treated with a non-inhibiting SSRI, patients on a CYP2C19-inhibiting SSRI had an increased risk of ischemic events (hazard ratio [HR] 1.12; 95 % confidence interval [CI]: 1.01 to 1.24), which was more pronounced in patients greater than or equal to 65 years (HR 1.22; 95 % CI: 1.00 to 1.48). The HR for major bleeding was 0.76 (95 % CI: 0.50 to 1.17). The authors concluded that the findings from this large, population-based study suggested
that being treated with a CYP2C19-inhibiting SSRI when initiating clopidogrel may be associated with slight decrease in effectiveness of clopidogrel.

**PGxOnePlus**

An UpToDate review on “Anxiety disorders in children and adolescents: Epidemiology, pathogenesis, clinical manifestations, and course” (Bennett and Walkup, 2017) states that “Studies suggest that children of parents with an anxiety disorder are at an increased risk of developing an anxiety disorder themselves. Twin studies have demonstrated significant familial aggregation among GAD, panic disorder, phobias, and obsessive-compulsive disorder. Most estimates of anxiety trait heritability in children are around 30 %, though as high as 50 to 60 % in some studies. These findings suggest that genetic factors play a large role in the development of these disorders relative to environmental factors. A meta-analysis of 13 cohorts (42,585 subjects) with social anxiety showed that genetic and non-shared environmental factors explain most of the variance for social anxiety disorder and social anxiety symptoms across age groups. Adult cohorts showed a higher contribution of non-shared environment and half the genetic contribution compared with younger patients. Some research suggests that the contribution of genetics to the development of anxiety disorders may change over the course of an individual’s development. Supporting a dynamic contribution of genetics, a study of 2,508 twins suggested an effect of some genes that are “turned on” in adolescence and others that are diminished throughout development. The overall variance accounted for by genetic contributions decreased from 72 % at ages 8 and 9 to 12 % at ages 19 and 20. It is unlikely that a single gene associated with one specific pathophysiological function is responsible for anxiety. Family and genetic studies suggest some people may have a genetic vulnerability to develop anxiety disorders generally. Genome Wide Association Studies (GWAS) have begun to look for specific candidate genes associated with anxiety disorders; however, these studies result in a large number of candidate genes with relatively small odds ratios or lack of statistical significance in genotype/phenotype associations, possibly due to the cumulative effect of many genes on the vulnerability for anxiety disorders. Genetic findings associated with anxiety disorders or anxiety-like behavior include a gene that promotes a corticotropin releasing hormone and a single nucleotide polymorphism (SNP) in the brain-derived neurotrophic factor (BDNF) gene ... Data from a study of 385 monozygotic and 486 dizygotic same-sex twin families assessed the children of twins and found evidence of a significant direct environmental transmission of anxiety from parent to child using structural equation modeling. There was no evidence of direct genetic transmission”. There is no mentioning of genetic testing in the “Summary and Recommendations” section.
It is also interesting to note that Health Quality Ontario (2017) states that the Assurex GeneSight Psychotropic test is a pharmacogenomic panel that provides clinicians with a report to guide medication selection that is unique to each patient based on their individual genetic profile. However, it is uncertain whether guided treatment using GeneSight is effective compared with unguided treatment (usual care). Health Quality Ontario concluded that there is uncertainty about the use of GeneSight Psychotropic pharmacogenomic genetic panel to guide medication selection. It was associated with improvements in some patient outcomes, but not others. As well, the confidence in these findings is low because of limitations in the body of evidence.

An UpToDate review on “Genetic factors in the pathogenesis of hypertension” (Ehret, 2017) states that “The use of genome-wide association studies, which examine hundreds of thousands of single-nucleotide polymorphisms (SNPs) in large cohorts, has improved the understanding of blood pressure genomics and has demonstrated the presence of clearly reproducible blood pressure loci. However, these loci have so far only explained a small proportion of the total blood pressure heritability. There are approximately 80 published blood pressure loci.” There is no mentioning of genetic testing in the “Summary and Recommendations” section.

Bonfiglio et al (2017) stated that gastro-esophageal reflux disease (GERD), the regurgitation of gastric acids often accompanied by heartburn, affects up to 20 % of the general population. Genetic predisposition is suspected from twin and family studies but gene-hunting efforts have so far been scarce and no conclusive genome-wide study has been reported. These researchers exploited data available from general population samples, and studied self-reported reflux symptoms in relation to genome-wide single nucleotide polymorphism (SNP) genotypes. The authors reported a large-scale genetic study of GERD, and highlighted genes and pathways that contribute to further our understanding of its pathogenesis and therapeutic opportunities.

An UpToDate review on “Approach to refractory gastroesophageal reflux disease in adults” (Fass, 2017) states that “Proton pump inhibitors are metabolized through the hepatic cytochrome system (specifically the CYP2C isoenzyme). As a result, genetically determined variability in the processes underlying drug metabolism may influence their efficacy. Patients with rapid metabolism of PPIs may have a decreased effect on gastric acidity. On the other hand, CYP2C is absent in about 3 % of Caucasian patients and in substantially higher numbers of Asians (greater than 10 %), potentially leading to greater suppression of gastric acidity”
Genotyping of Interferon-Lambda 3 (IFNL3) for Prediction of Virological Response to Pegylated-Interferon-Alpha and Ribavirin Combination Therapy

According to the Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for “IFNL3 (IL28B) genotype and peginterferon alpha based regimens”, Muir and colleagues (2014) stated that Peg-interferon-alpha and ribavirin (Peg-IFNα/RBV)-based regimens are the mainstay for the treatment of hepatitis C virus (HCV) genotype 1 infection. For patients treated with Peg-IFNα and RBV alone, interferon-lambda 3 (IFNL3) genotype is the strongest pre-treatment predictor of HCV treatment response. In the intention-to-treat analysis of the original discovery cohort with rs12979860, Caucasian patients with CC genotype were more likely than those with CT or TT genotype to have undetectable serum viral RNA by week 4 (28 % versus 5 % and 5 %, respectively; p < 0.0001) and to achieve sustained virologic response (SVR) (69 % versus 33 % and 27 %, respectively; p < 0.0001). Similar patterns were observed in Hispanic and African-American patients in this cohort; HCV treatment is associated with significant side effects, and the likelihood of response treatment influenced shared decision-making between clinicians and patients about initiating treatment.

Ishiguro and associates (2015) evaluated IFNL3 polymorphisms in response-guided Peg-IFNα/RBV therapy for genotype 2 (G2) chronic HVC infection. Between January 2006 and June 2012, a total of 180 patients with chronic infections of G2 HCV were treated with response-guided Peg-IFNα/RBV therapy. The treatment duration was 24 weeks for patients who achieved rapid virologic response (RVR), and 36 or 48 weeks for patients who did not. Then, the impact of the IFNL3 single nucleotide polymorphism (SNP) genotype (TT/non-TT at rs8099917) on treatment outcomes was evaluated in the 180 patients, and between patients infected with either HCV sub-genotype 2a or 2b. Of the 180 patients evaluated, 111 achieved RVR, while the remaining 69 patients did not. In RVR patients, the SVR rate was 96.4 %, and the IFNL3 genotype did not influence the SVR rate (96.6 % versus 95.8 % in IFNL3 genotype TT versus non-TT). However, in non-RVR patients, the SVR rate decreased to 72.5 % (p < 0.0001), and this rate was significantly different between the IFNL3 genotype TT and non-TT groups (80.0 % versus 42.9 %, p = 0.0146). Multi-variate regression analysis in non-RVR patients identified the IFNL3 genotype TT as the only baseline-significant factor associated with SVR (OR = 5.39, 95 % CI: 1.29 to 22.62; p = 0.0189). In analysis according to HCV sub-genotype, no significant difference in the SVR rate was found between HCV sub-genotypes 2a and 2b. The authors concluded that in response-guided Peg-IFNα/RBV combination therapy for chronically HCV G2-infected patients, the impact of the IFNL3 genotype on SVR was limited to non-RVR patients.
Haj-Sheykholeslami and co-workers (2015) evaluated the efficacy of Peg-IFN in chronic HCV patients in relation to IFNL polymorphisms. This study enrolled patients with chronic HCV referred to the Tehran Blood Transfusion Hepatitis Clinic in 2011. Patients were included in the study if they had no concomitant hepatic illness, were negative for human immunodeficiency virus (HIV) antibodies, and had no prior history of treatment with any type of Peg-IFN. Patients were treated with 180 μg Peg-IFNα-2a (Pegaferon) weekly and 800 to 1,200 mg ribavirin daily for 24 or 48 weeks depending on weight and HCV genotype. Blood samples were collected from patients to obtain DNA for determination of IFNL rs12979860 and rs8099917 polymorphisms. The virologic response in patients was then evaluated and compared between the different IFNL genotypes. A total of 152 patients with a mean age of 41.9 ± 10.0 years were included in the study, of which 141/152 were men (92.8 %). The most frequent HCV genotype was type-1, infecting 93/152 (61.2 %) patients; SVR was achieved in 81.9 % of patients with HCV genotype-1 and 91.1 % of patients with HCV genotype-3. Treatment success was achieved in 91.2 % (52/57) of patients with the IFNL rs12979860 CC genotype and 82.1 % (78/95) in those with other genotypes. Similar treatment response rates were also observed in patients with rs8099917 TT (39/45; 86.7 %) and non-TT (61/68; 89.7 %) genotypes. Uni-variate analyses identified the following factors that influenced treatment response for inclusion in a multi-variate analysis: age, HCV RNA level, stage of liver fibrosis, rs12979860 CC genotype, and aspartate transaminase level. A logistic regression analysis revealed that only the rs12979860 CC genotype was significantly associated with achievement of SVR (OR = 6.2; 95 % CI: 1.2 to 31.9; p = 0.03). The authors concluded that the rs12979860 CC genotype was associated with SVR in patients receiving Peg-IFNα/RBV therapy, however, the SVR rate in other rs12979860 genotypes was also relatively high.

Nakamoto and colleagues (2017) noted that genetic variation near the IFNL3 is known to be associated with response to Peg-IFNα/RBV therapy in patients with chronic HCV infection, which is often accompanied by hepatic steatosis. These researchers examined if this genetic variation is associated with host lipids and treatment response. A total of 101 Japanese patients who had undergone liver biopsy before treatment with Peg-IFNα/RBV therapy for HCV genotype 1b infection were retrospectively analyzed for association between IFNL3 genotypes (rs8099917) and clinical factors including histopathological features of the liver. The presence of greater than 5 % steatosis in the liver specimen was defined as hepatic steatosis. A total of 40 patients (40 %) had liver steatosis before therapy. Patients with IFNL3 minor genotype (non-TT) showed lower low-density lipoprotein cholesterol level (p = 0.0045), higher γ-glutamyl transpeptidase level (p = 0.0003) and higher prevalence of hepatic steatosis (p = 0.0002). Advanced fibrosis [OR 4.63, p = 0.03] and IFNL3 major genotype (OR 0.13, p = 0.001) were 2 independent factors for determining the presence of hepatic steatosis. Among the
factors associated with SVR, IFNL3 genotype was the most significant predictor, as per multi-variate analysis. The authors concluded that these findings confirmed that IFNL3 genotype was associated with hepatic steatosis as well as IFN response.

**UrSure Tenofovir Quantification Test**

Koenig and associates (2017) noted that tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) is approved for pre-exposure prophylaxis (PrEP) against HIV infection. Adherence is critical for the success of PrEP, but current adherence measurements are inadequate for real-time adherence monitoring. These researchers developed and validated a urine assay to measure tenofovir (TFV) to objectively monitor adherence to PrEP. These investigators developed a urine assay using high-performance liquid chromatography (HPLC) coupled to tandem mass spectrometry (MS) with high sensitivity/specificity for TFV that allowed these researchers to determine TFV concentrations in log10 categories between 0 and 10,000 ng/ml. They validated the assay in 3 cohorts:

1. HIV-positive subjects with undetectable viral loads on a TDF/FTC-based regimen,

2. healthy HIV-negative subjects who received a single dose of TDF/FTC, and

3. HIV-negative subjects receiving daily TDF/FTC as PrEP for 24 weeks.

The urine assay detected TFV with greater sensitivity than plasma-based measures and with a window of measurements within 7 days of the last TDF/FTC dose. Based on the urine log-linear clearance after the last dose and its concordance with all detectable plasma levels, a urine TFV concentration of greater than 1,000 ng/ml was identified as highly predictive of the presence of TFV in plasma at greater than 10 ng/ml. The urine assay was able to distinguish high and low adherence patterns within the last 48 hours (greater than 1,000 ng/ml versus 10 to 1,000 ng/ml), as well as non-adherence (less than 10 ng/ml) extended over at least 1 week prior to measurement. The authors provided
proof of concept that a semi-quantitative urine assay measuring levels of TFV could be further developed into a point-of-care (POC) test and be a useful tool to monitor adherence to PrEP.

Cressey and colleagues (2017) stated that TDF is key component of PrEP and anti-retroviral therapy (ART) for HIV, but existing tools to monitor drug adherence are often inaccurate. Detection of TFV in accessible biological samples, such as finger-prick blood, urine or oral fluid samples could be a novel objective measure of recent TDF adherence. To measure TFV concentrations associated with different levels of TDF adherence, these researchers designed a randomized clinical trial to evaluate the blood, urine and oral fluid concentrations of TFV in adults with perfect, moderate, and low drug adherence. This is a randomized, open-label, clinical pharmacokinetic study of TFV in healthy adult volunteers without HIV or hepatitis B infection in Thailand. Consenting, eligible participants are randomized (1:1:1) among 3 groups to receive a controlled number of TDF (300 mg) doses in a combination pill with emtricitabine (FTC, 200 mg) for 6 weeks. Participants in Group 1 receive a single TDF/FTC tablet once-daily (perfect adherence); Group 2 receive a single TDF/FTC tablet 4 times/week (moderate adherence); and Group 3 receive a single TDF/FTC tablet 2 times/week (low adherence). Blood, plasma, urine and oral fluid samples are collected for drug measurement during 3 study phases:

1. (initial 6-week treatment phase;

II. intensive 24-hour blood sampling phase after 6 weeks;

III. 4-week washout phase.

1. (initial 6-week treatment phase;
2. intensive 24-hour blood sampling phase after 6 weeks;
3. 4-week washout phase.

A total of 30 adults with evaluable pharmacokinetic samples (10 per group) will be enrolled [based on ensuring 25 % precision in pharmacokinetic parameter estimates]. Pre-dose drug concentrations during the treatment phase will be descriptive and comparisons between groups performed using a Kruskal-Wallis test. A non-
compartmental pharmacokinetic analysis will be performed on the intensive sampling data at week 7 and the time course of TFV washout in the difference biological matrices will be reported based on the detected concentrations following drug cessation. The authors concluded that the findings of this randomized trial will define the target concentration thresholds of TFV in blood, urine and oral fluid that can distinguish between different levels of TDF adherence. Such adherence “benchmarks” can be applied to real-time drug testing and novel POC tests to identify individuals with poor PrEP or ART adherence. Moreover, they stated that the introduction of scalable TFV-based POC tests may allow rapid identification of patients struggling with PrEP or ART adherence, in order to develop and implement targeted adherence interventions. They noted that improving adherence to both PrEP and ART will help reduce and prevent HIV transmission, while also preserving the use an important drug in the efforts to end the HIV/AIDS epidemic. The enrollment to this clinical trial is by invitation (last updated October 26, 2017).

Current guidelines on preexposure prophylaxis have no recommendations for urine tenofovir monitoring. Also, no recommendations for urine tenofovir monitoring are included in the PrEP prescribing information. Additional studies demonstrating the clinical utility of urine tenofovir monitoring are needed, as well as comparisons of urine drug monitoring with other established methods of improving PrEP compliance.

**INFINITI Neural Response Panel**

On June 04, 2018, the FDA granted Breakthrough Device designation to the INFINITI Neural Response Panel by AutoGenomics, Inc., which indicates that the FDA has agreed to prioritize and expedite their review of the panel test. The INFINITI Neural Response Panel is an in vitro, qualitative, automated, microarray–based molecular diagnostic test for the identification of patients who may be at risk for opioid dependency. The panel is designed to identify mutations in 16 genes (ABCB1, COMT, DAT1, DBH, DOR, DRD1, DRD2, DRD4, GABA, GAL, HTR2A, HTTLPR, MTHFR, MUOR, OPRK1, and OPRM1) involved in the brain reward pathways that are associated with increased risk of opioid use disorder, and is intended for use by physicians as an aid for safe and effective pain management.

Two studies suggesting that a new genetic test could potentially help predict which patients are at risk of abusing prescription opioids were first presented at the 69th AACC Annual Scientific Meeting & Clinical Lab Expo in San Diego in 2017. In the first study, Donaldson et al (2017; Prescient Medicine) stated over 116 million people worldwide have chronic pain and prescription dependence. In the US, opioids account for the
majority of overdose deaths, and in 2014, almost 2 million Americans abused or were dependent on prescription opioids. Genetic factors may play a key role in opioid prescription addiction. The authors describe genetic variations between opioid addicted and non-addicted populations and derive a predictive model determining risk of opioid addiction. This case cohort study compares the frequency of 16 single nucleotide polymorphisms involved in the brain reward pathways in patients with and without opioid addiction. Data from 37 patients with prescription opioid or heroin addiction and 30 age and gender matched controls were used to design the predictive score. The predictive score was then tested on an additional 138 samples to determine generalizability. Results for Method Derivation of Observed data: ROC statistic=0.92, sensitivity=82% (95% CI: 66-90), specificity=75% (95% CI:56-87). TreeNet “learn” data: ROC statistic=0.92, sensitivity=92%, specificity=90%, precision=92%, and overall correct=91%. Results of Generalizability data: Sensitivity=97% (95% CI: 90 to 100), specificity=87% (95% CI: 86 to 93), positive likelihood ratio=7.3 (95% CI: 4.0 to 13.5), and negative likelihood ratio=0.03 (95% CI: 0.01 to 0.13). This negative likelihood ratio can be used as evidence based measure to exclude patients with a high risk of opioid addiction or substance use disorder. By identifying patients with a lower risk for opioid addiction, our model may inform therapeutic decisions. The authors further state that these types of predictive risk scores may also foster tailored medical detoxification regimens and reduce drug related adverse events for patients. Yet the use of genetic algorithms to determine predictive risk scores is still a relatively new science an prospective, longitudinal studies are needed to better define the breadth of the test’s importance.

In the second clinical study by Chang et al. (2017; AutoGenomics) assessed the risk of developing prescription opioid addiction with a multi-variant addiction panel involved in the mesolimbic dopamine system. The authors genotyped samples for 16 single nucleotide polymorphisms (SNPs) involved in the brain reward pathways from 70 patients diagnosed with prescription opioid/heroin addiction and 68 normal control patients with a multiplexed film-based microarray technology. The addiction panel targets 16 mutations: 5-HTR2A (rs7997012), 5-HTTLPR (rs25531), COMT (rs4680), DRD2 (rs1800497), DRD1 (rs4532), DRD4 (rs3758653), DAT1 (rs6347), DBH (rs1611115), MTHFR (rs1801133), OPRK1 (rs1051660), GABA (rs211014), OPRM1 (rs1799971), MUOR (rs9479757), GAL (rs948854), DOR (rs2236861) and ATP-BCT (rs1045642). The genotyping data were subjected to a class predication model building with 10-fold cross validation for testing. A risk score from 1 to 100 was further computed with a score over 52 representing an elevated risk of addiction. The receiver operating characteristic (ROC) for the model was 0.78 with 76% sensitivity (95% CI: 84 - 85) and 72% specificity (95% CI: 60 - 82). PPV is 74% and NPV is 74%. Fifty-Three of 70 (75.7%) addicts and 19 of 68 (27.9%) normal controls showed an addiction risk score over 52. (X2=31.55;df=1;P<.05).
The authors concluded that the prediction algorithm with this multi-variant genetic panel can be used for prescription opioid addiction risk assessment. By identifying patients with high risk to prescription opioid addiction along with mutation status of cytochrome p450 genes involved in therapeutics, it may provide information to physicians to improve therapeutic decisions in pain management and prevent abuse and addiction.

**Snapshot Oral Fluid Compliance**

Snapshot Oral Fluid Compliance test by Ethos Laboratories is a prescription drug monitoring enzyme immunoassay of oral fluid samples that tests 35 or more drugs with liquid chromatography mass spectrometry (LC-MS/MS) for patient-compliance and drug to drug interactions for prescribed medications. Results are summarized in a specialized report (i.e., Ethos Snapshot).

Richter et al (2019) stated nonadherence to antihypertensive drugs therapy is known to be a serious issue in hypertension treatment. Liquid chromatography (LC) coupled to mass spectrometry (MS) was shown to allow the assessment of such nonadherence in blood and urine sample. However, their sampling may represent a logistical challenge and are often not favored by the patients. The authors questioned whether oral fluid (OF) might be an easier accessible alternative matrix for adherence monitoring of cardiovascular drugs (CD). A qualitative method for adherence monitoring of 78 commonly prescribed cardiovascular drugs in OF using LC high-resolution MS (LC-HRMS/MS) was therefore developed, validated, and used to study the presence of antihypertensive medication in OF. Selectivity, ion suppression and enhancement due coeluting analytes, carry over, limits of detection (LOD), limits of identification (LOI), recovery (RE), matrix effects (ME), and process efficiency (PE) were investigated. For demonstrating applicability, over 50 OF samples were investigated and data were compared to findings in blood and urine. Selectivity in OF was given for all compounds via their MS2 spectra and no total suppression of signals could be observed. Determined LOI in OF for ten analytes was higher than the given therapeutic plasma concentration. Furthermore, RE, ME, and PE were in acceptable ranges for more than 65% of the compounds. In total, 208 prescriptions of CD to 57 patients were analyzed and demonstrated the suitability of for adherence monitoring in principle. OF was comparable to plasma regarding the drug categories and the frequencies of hits, except for acidic compounds but more hits could be found in urine samples. A analytical method using OF as analytical matrix was successfully developed. The authors concluded that application showed that it might be a suitable alternative for adherence monitoring of selected drugs in the future, particularly those having no acidic function.
OncolyticAssuranceRX

OncolyticAssuranceRX, by Firstox Laboratories, LLC., is a prescription drug monitoring test that monitors adherence to one or more oral oncology drug(s) and substances through a proprietary liquid chromatography mass spectrometry (LC-MS/MS) test combined with a minimally invasive finger stick collection, and provides a quantitative report with steady-state range for the prescribed drug(s) when detected.

Janssen et al (2019) stated a liquid chromatography-tandem mass spectrometry assay was developed and validated for the nine oral anticancer agents alectinib, cobimetinib, lenvatinib, nintedanib, osimertinib, palbociclib, ribociclib, vismodegib and vorinostat in order to support therapeutic drug monitoring (TDM). The assay was based on reversed-phase chromatography coupled with tandem mass spectrometry operating in the positive ion mode. The assay was validated based on the guidelines on bioanalytical methods by the US Food and Drug Administration and European Medicines Agency. The method was validated over a linear range of 10-200 ng/mL for alectinib, lenvatinib, nintedanib and vismodegib; 50-1000 ng/mL for cobimetinib and palbociclib; 100-2000 ng/mL for osimertinib; 5.00-100 ng/mL for ribociclib; 25-500 ng/mL for vorinostat. Intra-assay and inter-assay bias was within ±20% for all analytes at the lower limit of quantification and within ±15% at remaining concentrations. Stability experiments showed that osimertinib is unstable in the biomatrix and should be shipped on dry-ice and stored at -20 °C until analysis. All other compounds were stable in the biomatrix. The authors concluded that the described TDM method was successfully validated and applied for TDM in patients treated with these KIs.

van Nuland M et al (2019) stated a liquid chromatography-mass spectrometry assay was developed and validated for simultaneous quantification of anti-hormonal compounds abiraterone, anastrozole, bicalutamide, Δ(4)-abiraterone (D4A), N-desmethyl enzalutamide, enzalutamide, Z-endoxifen, exemestane and letrozole for the purpose of therapeutic drug monitoring (TDM). Plasma samples were prepared with protein precipitation. Analyses were performed with a triple quadrupole mass spectrometer operating in the positive and negative ion-mode. The validated assay ranges from 2 to 200 ng/mL for abiraterone, 0.2-20 ng/mL for D4A, 10-200 ng/mL for anastrozole and letrozole, 1-20 ng/mL for Z-endoxifen, 1.88-37.5 ng/mL for exemestane and 1500-30,000 ng/mL for enzalutamide, N-desmethyl enzalutamide and bicalutamide. Due to low sensitivity for exemestane, the final extract of exemestane patient samples should be concentrated prior to injection and a larger sample volume should be prepared for exemestane patient samples and QC samples to obtain adequate sensitivity. Furthermore, we observed a batch-dependent stability for abiraterone in plasma at room temperature and therefore samples should be shipped on ice. The authors
concluded that this newly validated method has been successfully applied for routine TDM of anti-hormonal drugs in cancer patients.

Cardoso et al (2018) stated A sensitive and selective method of high performance liquid chromatography (HPLC) coupled to tandem mass spectrometry (MS/MS) has been developed for the simultaneous quantification of six anticancer protein kinase inhibitors (PKIs), dabrafenib, trametinib, vemurafenib, cobimetinib, pazopanib, regorafenib, and two active metabolites (regorafenib-M2 and regorafenib-M5) in human plasma. Plasma protein precipitation with methanol enables the sample extraction of 100 μL aliquot of plasma. Analytes are detected by electrospray triple-stage quadrupole mass spectrometry and quantified using the calibration curves with stable isotope-labeled internal standards. The method was validated based on FDA recommendations, including assessment of extraction yield (74-104%), matrix effects, analytical recovery (94-104%) with low variability (<15%). The method is sensitive (lower limits of quantification within 1 to 200 ng/mL), accurate (intra- and inter-assay bias: -0.3% to +12.7%, and -3.2% to +6.3%, respectively) and precise (intra- and inter-assay CVs within 0.7-7.3% and 2.5-8.0%, respectively) over the clinically relevant concentration range (upper limits of quantification 500 to 100,000 ng/mL). The authors concluded that this method is applied in their laboratory for both clinical research programs and routine therapeutic drug monitoring service of PKIs.

FGFR Testing for pemigatinib (Pemazyre) therapy for advanced cholangiocarcinoma

Abou-Alfa et al (2020) stated fibroblast growth factor receptor (FGFR) 2 gene alterations are involved in the pathogenesis of cholangiocarcinoma. Pemigatinib is a selective, potent, oral inhibitor of FGFR1, 2, and 3. This study evaluated the safety and antitumour activity of pemigatinib in patients with previously treated, locally advanced or metastatic cholangiocarcinoma with and without FGFR2 fusions or rearrangements. In this multicentre, open-label, single-arm, multicohort, phase 2 study (FIGHT-202), patients aged 18 years or older with disease progression following at least one previous treatment and an Eastern Cooperative Oncology Group (ECOG) performance status of 0-2 recruited from 146 academic or community-based sites in the USA, Europe, the Middle East, and Asia were assigned to one of three cohorts: patients with FGFR2 fusions or rearrangements, patients with other FGF/FGFR alterations, or patients with no FGF/FGFR alterations. All enrolled patients received a starting dose of 13·5 mg oral pemigatinib once daily (21-day cycle; 2 weeks on, 1 week off) until disease progression, unacceptable toxicity, withdrawal of consent, or physician decision. The primary endpoint was the proportion of patients who achieved an objective response.
among those with FGFR2 fusions or rearrangements, assessed centrally in all patients who received at least one dose of pemigatinib. This study is registered with ClinicalTrials.gov, NCT02924376, and enrolment is completed. Between Jan 17, 2017, and March 22, 2019, 146 patients were enrolled: 107 with FGFR2 fusions or rearrangements, 20 with other FGF/FGFR alterations, 18 with no FGF/FGFR alterations, and one with an undetermined FGF/FGFR alteration. The median follow-up was 17·8 months (IQR 11·6-21·3). 38 (35·5% [95% CI 26·5-45·4]) patients with FGFR2 fusions or rearrangements achieved an objective response (three complete responses and 35 partial responses). Overall, hyperphosphataemia was the most common all-grade adverse event irrespective of cause (88 [60%] of 146 patients). 93 (64%) patients had a grade 3 or worse adverse event (irrespective of cause); the most frequent were hypophosphataemia (18 [12%]), arthralgia (nine [6%]), stomatitis (eight [5%]), hyponatraemia (eight [5%]), abdominal pain (seven [5%]), and fatigue (seven [5%]). 65 (45%) patients had serious adverse events; the most frequent were abdominal pain (seven [5%]), pyrexia (seven [5%]), cholangitis (five [3%]), and pleural effusion (five [3%]). Overall, 71 (49%) patients died during the study, most frequently because of disease progression (61 [42%]); no deaths were deemed to be treatment related. The authors concluded that these data support the therapeutic potential of pemigatinib in previously treated patients with cholangiocarcinoma who have FGFR2 fusions or rearrangements.

**EZH2 Testing for tazemetostat (Tazverik) therapy for follicular lymphoma**

The efficacy of Tazverik was evaluated in two open-label, single-arm cohorts (cohort 4: EZH2-mutated follicular lymphoma and cohort 5: EZH2 wild-type follicular lymphoma) of a multi-center study (Study E7438-G000-101, NCT01897571) in patients with histologically confirmed follicular lymphoma after at least two prior systemic therapies. Patients were required to have ECOG PS of 0–2 and were enrolled based on EZH2 mutation status. EZH2 mutations were identified prospectively using formalin-fixed, paraffin-embedded tumor samples, which were centrally tested using the cobas EZH2 Mutation Test; the cobas EZH2 Mutation test is designed to detect the following mutations: Y646X [S,H,C], Y646F, Y646N, A682G, and A692V. Patients received TAZVERIK 800 mg orally twice daily until confirmed disease progression or unacceptable toxicity. Tumor response assessments were performed every 8 weeks through Week 24 and then every 12 weeks. The major efficacy outcome measures were overall response rate and duration of response according to the International Working Group Non-Hodgkin Lymphoma (IWG-NHL) criteria as assessed by Independent Review Committee. Median duration of follow-up was 22 months (range 3 months to 44 months) for patients with EZH2 MT positive tumors and 36 months (range 32 months to 39 months) for patients.
whose tumors did not have an EZH2 mutation detected. A total of 99 patients were enrolled, including 45 patients whose tumors had one of these EZH2 mutations (mutant) and 54 patients whose tumors did not have one of these mutations (wild-type). Among the 45 patients with EZH2 mutant follicular lymphoma, median age was 62 years (range 38 to 80), 58% were female, 42% had early progression following front-line therapy (POD24), and all had an ECOG PS of 0 or 1. Race was reported in 84% of patients; of these patients, 82% were White. Based on the cobas EZH2 Mutation test, 36%, 29%, 27%, 11%, and 2% of patients had the following mutations: Y646X [S,H,C], Y646F, Y646N, A682G, and A692V, respectively. The median number of lines of prior systemic therapy was 2 (range 1 to 11), with 49% refractory to rituximab, 49% refractory to their last therapy, and 9% had received prior stem cell transplant. Among the 54 patients with EZH2 wild-type follicular lymphoma, median age was 61 years (range 36 to 87), 63% were male, 59% had POD24, and 91% had an ECOG PS of 0 or 1. Race was reported in 57% of patients; of these patients, 48% were White and 3% were Asian. The median number of lines of prior systemic therapy was 3 (range 1 to 8), with 59% refractory to rituximab, 41% refractory to their last therapy, and 39% had received prior stem cell transplant. The approval of Tazverik was based upon the efficacy in 95 patients (42 EZH2 Mutant, 53 EZH2 WildType) who had received at least 2 prior systemic therapies. The overall response rate in 42 patients with EZH2-mutated follicular lymphoma was 69% (95% confidence interval [CI] = 53%–82%), with 12% of patients having a complete response and 57%, a partial response. Median duration of response in these patients was 10.9 months (95% CI = 7.2–not evaluable). The overall response rate in 53 patients with EZH2 wild-type follicular lymphoma was 34% (95% CI = 22%–48%), with 4% of patients having a complete response and 30%, a partial response. Median duration of response was 13 months (95% CI = 5.6–not evaluable). The most commonly reported (≥ 20%) adverse reactions included fatigue, upper respiratory tract infection, musculoskeletal pain, nausea, and abdominal pain. Serious adverse reactions occurred in 30% of patients, most often from infection. Second primary malignancy was the most common reason for treatment discontinuation (2% of patients). The prescribing information includes a warning and precaution for secondary malignancies. The recommended tazemetostat dose is 800 mg taken orally twice daily with or without food (Epizyme 2020).

Table:CPT Codes / HCPCS Codes / ICD-10 Codes
Information in the [brackets] below has been added for clarification purposes. Codes requiring a 7th character are represented by "+":
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<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotyping for CYP2C19:</td>
<td></td>
</tr>
<tr>
<td>Code</td>
<td>Code Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>CPT codes covered if selection criteria are met:</td>
<td></td>
</tr>
<tr>
<td>CPT codes not covered for indications listed in the CPB:</td>
<td></td>
</tr>
<tr>
<td>ICD-10 codes covered if selection criteria are met:</td>
<td></td>
</tr>
<tr>
<td>I25.10 - I25.9</td>
<td>Chronic ischemic heart disease</td>
</tr>
<tr>
<td>Genotyping for CYP2D6:</td>
<td></td>
</tr>
<tr>
<td>CPT codes covered if selection criteria are met:</td>
<td></td>
</tr>
<tr>
<td>0028U</td>
<td>CYP2D6 (cytochrome P450, family 2, subfamily D, polypeptide 6) (eg, drug metabolism) gene analysis, copy number variants, common variants with reflex to targeted sequence analysis</td>
</tr>
<tr>
<td>0071U</td>
<td>CYP2D6 (cytochrome P450, family 2, subfamily D, polypeptide 6) (eg, drug metabolism) gene analysis, full gene sequence (List separately in addition to code for primary procedure)</td>
</tr>
<tr>
<td>0072U</td>
<td>CYP2D6 (cytochrome P450, family 2, subfamily D, polypeptide 6) (eg, drug metabolism) gene analysis, targeted sequence analysis (ie, CYP2D6-2D7 hybrid gene) (List separately in addition to code for primary procedure)</td>
</tr>
<tr>
<td>0073U</td>
<td>CYP2D6 (cytochrome P450, family 2, subfamily D, polypeptide 6) (eg, drug metabolism) gene analysis, targeted sequence analysis (ie, CYP2D7-2D6 hybrid gene) (List separately in addition to code for primary procedure)</td>
</tr>
<tr>
<td>0074U</td>
<td>CYP2D6 (cytochrome P450, family 2, subfamily D, polypeptide 6) (eg, drug metabolism) gene analysis, targeted sequence analysis (ie, non-duplicated gene when duplication/multiplication is trans) (List separately in addition to code for primary procedure)</td>
</tr>
<tr>
<td>0075U</td>
<td>CYP2D6 (cytochrome P450, family 2, subfamily D, polypeptide 6) (eg, drug metabolism) gene analysis, targeted sequence analysis (ie, 5’ gene duplication/multiplication) (List separately in addition to code for primary procedure)</td>
</tr>
</tbody>
</table>
| 0076U        | CYP2D6 (cytochrome P450, family 2, subfamily D, polypeptide 6) (eg, drug metabolism) gene analysis, targeted sequence analysis (ie, 3’ gene duplication/
<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>J1800</td>
<td>Injection, propranolol HCl, up to 1 mg</td>
</tr>
<tr>
<td>E75.22</td>
<td>Gaucher disease [for persons with Gaucher's disease type 1 who are being considered for treatment with eligustat (Cerdelga)]</td>
</tr>
<tr>
<td>G10</td>
<td>Huntington's disease [Tetrabenazine (Xenazine) is indicated for the treatment of chorea associated with Huntington’s disease]</td>
</tr>
</tbody>
</table>

**Genotyping for cytochrome P450:**

No specific code

**CPT code not covered for indications listed in the CPB:**

<table>
<thead>
<tr>
<th>Code</th>
<th>Drug metabolism (adverse drug reactions and drug response), targeted sequence analysis (ie, CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4, CYP3A5, CYP4F2, SLCO1B1, VKORC1 and rs12777823)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0029U</td>
<td>Drug metabolism (warfarin drug response), targeted sequence analysis (ie, CYP2C9, CYP4F2, VKORC1, rs12777823)</td>
</tr>
<tr>
<td>0030U</td>
<td>CYP1A2 (cytochrome P450 family 1, subfamily A, member 2)(eg, drug metabolism) gene analysis, common variants (ie, *1F, *1K, *6, *7)</td>
</tr>
<tr>
<td>81230</td>
<td>CYP3A4 (cytochrome P450 family 3 subfamily A member 4) (eg, drug metabolism), gene analysis, common variant(s) (eg, *2, *22)</td>
</tr>
<tr>
<td>81232</td>
<td>DPYD (dihydropyrimidine dehydrogenase) (eg, 5-fluourouracil/5-FU and capecitabine drug metabolism), gene analysis, common variant(s) (eg, *2A, *4, *5, *6)</td>
</tr>
<tr>
<td>81346</td>
<td>TYMS (thymidylate synthetase) (eg, 5-fluourouracil/5-FU drug metabolism), gene analysis, common variant(s) (eg, tandem repeat variant)</td>
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</table>

**CPT codes related to the CPB:**

<table>
<thead>
<tr>
<th>Code</th>
<th>Molecular pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>G9143</td>
<td>Warfarin responsiveness testing by genetic technique using any method, any</td>
</tr>
</tbody>
</table>
### Table: CPT Codes / HCPCS Codes / ICD-10 Codes

*Information in the [brackets] below has been added for clarification purposes. Codes requiring a 7th character are represented by "+":*

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<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>number of specimen(s)</td>
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</tbody>
</table>

**Other HCPCS codes related to the CPB:**

<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>C9037</td>
<td>Injection, risperidone (perseris), 0.5 mg</td>
</tr>
<tr>
<td>J0640</td>
<td>Injection, leucovorin calcium, per 50 mg</td>
</tr>
<tr>
<td>J1630</td>
<td>Injection, haloperidol, up to 5 mg</td>
</tr>
<tr>
<td>J1631</td>
<td>Injection, haloperidol decanoate, per 50 mg</td>
</tr>
<tr>
<td>J2270</td>
<td>Injection, morphine sulfate, up to 10 mg</td>
</tr>
<tr>
<td>J2271</td>
<td>Injection, morphine sulfate, 100 mg</td>
</tr>
<tr>
<td>J2275</td>
<td>Injection, morphine sulfate (preservative-free sterile solution), per 10 mg</td>
</tr>
<tr>
<td>J2794</td>
<td>Injection, risperidone, long acting, 0.5 mg</td>
</tr>
<tr>
<td>J3310</td>
<td>Injection, perphenazine, up to 5 mg</td>
</tr>
<tr>
<td>J3360</td>
<td>Injection, diazepam, up to 5 mg</td>
</tr>
<tr>
<td>J8530</td>
<td>Cyclophosphamide, oral, 25 mg</td>
</tr>
<tr>
<td>J9070</td>
<td>Cyclophosphamide, 100 mg</td>
</tr>
<tr>
<td>J9190</td>
<td>Fluorouracil, 500 mg</td>
</tr>
<tr>
<td>J9206</td>
<td>Irinotecan, 20 mg</td>
</tr>
<tr>
<td>J9263</td>
<td>Injection, oxaliplatin, 0.5 mg</td>
</tr>
<tr>
<td>Q0175</td>
<td>Perphenazine, 4 mg, oral, FDA approved prescription anti-emetic, for use as a complete therapeutic substitute for an IV anti-emetic at the time of chemotherapy treatment, not to exceed a 48-hour dosage regimen</td>
</tr>
<tr>
<td>S0093</td>
<td>Injection, morphine sulfate, 500 mg (loading doe for infusion pump)</td>
</tr>
<tr>
<td>S0187</td>
<td>Tamoxifen citrate, oral, 10 mg</td>
</tr>
</tbody>
</table>

**ICD-10 codes not covered for indications listed in the CPB:**

<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>G30.0 - G30.9</td>
<td>Alzheimer's disease</td>
</tr>
</tbody>
</table>

**CYP2C19 polymorphisms testing for fluoxetine:**

**CPT codes not covered for indications listed in the CPB:**

<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
</table>

**UGT1A1 molecular assay:**

**CPT codes not covered for indications listed in the CPB:**

Proprietary
Table: CPT Codes / HCPCS Codes / ICD-10 Codes

Information in the [brackets] below has been added for clarification purposes. Codes requiring a 7th character are represented by "+":

<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
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</table>

Other HCPCS codes related to the CPB:

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>J9206</td>
<td>Irinotecan, 20 mg</td>
</tr>
</tbody>
</table>

ICD-10 codes not covered for indications listed in the CPB:

- C00.0 - C7A.8, C7B.1, C76.0 - C86.6, C88.4 - C94.32, C94.80 - C96.4, C96.6 - C96.9

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>D00.00 - D09.9</td>
<td>In situ neoplasms</td>
</tr>
</tbody>
</table>
### Table: CPT Codes / HCPCS Codes / ICD-10 Codes

*Information in the [brackets] below has been added for clarification purposes. Codes requiring a 7th character are represented by "+":*

<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>D45</td>
<td>Polycythemia vera</td>
</tr>
</tbody>
</table>

**VKORC1 polymorphism:**

CPT codes not covered for indications listed in the CPB:

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>81355</td>
<td>VKORC1 (vitamin K epoxide reductase complex, subunit 1) (eg, warfarin metabolism), gene analysis, common variant(s) (eg, -1639G&gt;A, c.173+1000C&gt;T)</td>
</tr>
</tbody>
</table>

**HLA-B*1502:**

No specific codes

**HLA-B*5701:**

No specific code

**ICD-10 codes covered if selection criteria are met:**

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>B20</td>
<td>Human immunodeficiency virus [HIV] disease</td>
</tr>
<tr>
<td>Z21</td>
<td>Asymptomatic human immunodeficiency virus [HIV] infection status</td>
</tr>
</tbody>
</table>

**Genotyping for apolipoprotein E (Apo E):**

No specific code

**Other CPT codes related to the CPB:**

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>88271 - 88275</td>
<td>Molecular cytogenetics</td>
</tr>
</tbody>
</table>

**ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):**

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>E71.30</td>
<td>Disorder of fatty-acid metabolism, unspecified</td>
</tr>
<tr>
<td>E75.5 - E75.6</td>
<td>Other and unspecified lipid storage disorders</td>
</tr>
<tr>
<td>E78.0 - E78.5, E78.70, E78.79 - E78.9</td>
<td>Disorders of lipoprotein metabolism and other lipidemias</td>
</tr>
<tr>
<td>E88.2</td>
<td>Lipomatosis, not elsewhere classified</td>
</tr>
</tbody>
</table>
Table: CPT Codes / HCPCS Codes / ICD-10 Codes

**Information in the [brackets] below has been added for clarification purposes. Codes requiring a 7th character are represented by "+":**

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<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>E88.89</td>
<td>Other specified metabolic disorders</td>
</tr>
</tbody>
</table>

**Genotyping for methylenetetrahydrofolate reductase (MTHFR):**

CPT codes not covered for indications listed in the CPB:

<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>81291</td>
<td>MTHFR (5,10-methylenetetrahydrofolate reductase) (eg, hereditary hypercoagulability) gene analysis, common variants (eg, 677T, 1298C)</td>
</tr>
</tbody>
</table>

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):

- C00.0 - C7A .8, C7B. 1, C76. 0 - C86. 6, Malignant neoplasms
- C88. 4 - C94. 32, C94. 80 - C96. 4, In situ neoplasms
- C96. 6 - C96. 9
- D00.00 - In situ neoplasms
<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>D09.9</td>
<td>Polycythemia vera</td>
</tr>
<tr>
<td><strong>D45</strong></td>
<td>Polycythemia vera</td>
</tr>
</tbody>
</table>

**Measurement of thromboxane metabolites in urine:**

CPT codes not covered for indications listed in the CPB:

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>84431</td>
<td>Thromboxane metabolite(s), including thromboxane if performed, urine</td>
</tr>
</tbody>
</table>

**BRAF V600E or V600K mutations (e.g., the THxID BRAF test):**

CPT codes covered if selection criteria are met:

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>81210</td>
<td>BRAF (B-Raf proto-oncogene, serine/threonine kinase) (eg, colon cancer, melanoma), gene analysis, V600 variant(s)</td>
</tr>
</tbody>
</table>

Other HCPCS codes related to the CPB:

Dabrafenib (Tafinlar); Vemurafenib (Zelboraf); Encorafenib (Braftovi), Cobimetinib (Cotellic), Binimetinib (Mektovi) - no specific code:

**J9271** | Injection, pembrolizumab, 1 mg |

ICD-10 codes covered by indications listed in the CPB:

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>C34.00 - C34.92</td>
<td>Malignant neoplasm of bronchus and lung [non-small cell lung cancer]</td>
</tr>
<tr>
<td>C43.0 - C43.9</td>
<td>Malignant melanoma of skin</td>
</tr>
<tr>
<td>C73</td>
<td>Malignant neoplasm of thyroid</td>
</tr>
</tbody>
</table>

**NS3 Q80K polymorphism, NS5A resistance-associated polymorphisms:**

CPT codes covered if selection criteria are met:

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>87900</td>
<td>Infectious agent drug susceptibility phenotype prediction using regularly updated genotypic bioinformatics [for persons with hepatitis C virus (HCV) genotype 1a infection being considered for treatment with simeprevir (Olysio)] [for persons with hepatitis C virus (HCV) genotype 1, 3 and 4 infections being considered for treatment with daclatasvir (Daklinza) or elbasvir and grazoprevir (Zepatier)]</td>
</tr>
<tr>
<td>87902</td>
<td>Infectious agent genotype analysis by nucleic acid (DNA or RNA); Hepatitis C virus [for persons with hepatitis C virus (HCV) genotype 1a infection being considered for treatment with simeprevir (Olysio)] [for persons with hepatitis C virus (HCV) genotype 1, 3 and 4 infections being considered for treatment with daclatasvir (Daklinza) or elbasvir and grazoprevir (Zepatier)]</td>
</tr>
</tbody>
</table>

ICD-10 codes covered if selection criteria are met:

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>B17.10 - B17.11</td>
<td>Acute hepatitis C without/with hepatic coma [genotype 1, 1a, 3, 4 infections]</td>
</tr>
</tbody>
</table>
### Information in the [brackets] below has been added for clarification purposes. Codes requiring a 7th character are represented by "+":

<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>B18.2</td>
<td>Chronic viral hepatitis C [genotype 1, 1a, 3, 4 infections]</td>
</tr>
<tr>
<td>B19.20 - B19.21</td>
<td>Unspecified viral hepatitis C [genotype 1, 1a, 3, 4 infections]</td>
</tr>
<tr>
<td>Z22.52</td>
<td>Carrier of viral hepatitis C [genotype 1, 1a, 3, 4 infections]</td>
</tr>
</tbody>
</table>

**BRACAnalysis CDx, FoundationOne CDx:**

CPT codes covered if selection criteria are met:

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0172U</td>
<td>Oncology (solid tumor as indicated by the label), somatic mutation analysis of BRCA1 (BRCA1, DNA repair associated), BRCA2 (BRCA2, DNA repair associated) and analysis of homologous recombination deficiency pathways, DNA, formalin-fixed paraffin-embedded tissue, algorithm quantifying tumor genomic instability score</td>
</tr>
<tr>
<td>81162</td>
<td>BRCA1, BRCA2 (breast cancer 1 and 2) (eg, hereditary breast and ovarian cancer) gene analysis; full sequence analysis and full duplication/deletion analysis</td>
</tr>
<tr>
<td>81163</td>
<td>BRCA1 (BRCA1, DNA repair associated), BRCA2 (BRCA2, DNA repair associated) (eg, hereditary breast and ovarian cancer) gene analysis; full sequence analysis</td>
</tr>
<tr>
<td>81165</td>
<td>BRCA1 (BRCA1, DNA repair associated) (eg, hereditary breast and ovarian cancer) gene analysis; full sequence analysis</td>
</tr>
<tr>
<td>81212</td>
<td>BRCA1 (BRCA1, DNA repair associated), BRCA2 (BRCA2, DNA repair associated) (eg, hereditary breast and ovarian cancer) gene analysis; 185delAG, 5385insC, 6174delT variants</td>
</tr>
<tr>
<td>81215</td>
<td>BRCA1 (BRCA1, DNA repair associated) (eg, hereditary breast and ovarian cancer) gene analysis; known familial variant</td>
</tr>
<tr>
<td>81216</td>
<td>BRCA2 (BRCA2, DNA repair associated) (eg, hereditary breast and ovarian cancer) gene analysis; full sequence analysis</td>
</tr>
<tr>
<td>81217</td>
<td>BRCA2 (BRCA2, DNA repair associated) (eg, hereditary breast and ovarian cancer) gene analysis; known familial variant</td>
</tr>
</tbody>
</table>

CPT codes not covered for indications listed in the CPB:

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>81164</td>
<td>BRCA1 (BRCA1, DNA repair associated), BRCA2 (BRCA2, DNA repair associated) (eg, hereditary breast and ovarian cancer) gene analysis; full duplication/deletion analysis (ie, detection of large gene rearrangements)</td>
</tr>
<tr>
<td>81166</td>
<td>BRCA1 (BRCA1, DNA repair associated) (eg, hereditary breast and ovarian cancer) gene analysis; full duplication/deletion analysis (ie, detection of large gene rearrangements)</td>
</tr>
<tr>
<td>81167</td>
<td>BRCA2 (BRCA2, DNA repair associated) (eg, hereditary breast and ovarian cancer) gene analysis; full duplication/deletion analysis (ie, detection of large gene rearrangements)</td>
</tr>
<tr>
<td>Code</td>
<td>Code Description</td>
</tr>
<tr>
<td>----------</td>
<td>-----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>C25.0 - C25.9</td>
<td>Malignant neoplasm of pancreas</td>
</tr>
<tr>
<td>C48.0 - C48.8</td>
<td>Malignant neoplasm of retroperitoneum and peritoneum</td>
</tr>
<tr>
<td>C50.011 - C50.929</td>
<td>Malignant neoplasm of breast [HER2-negative]</td>
</tr>
<tr>
<td>C56.1 - C56.9</td>
<td>Malignant neoplasm of ovary</td>
</tr>
<tr>
<td>C57.00 - C57.02</td>
<td>Malignant neoplasm of fallopian tube</td>
</tr>
<tr>
<td>C61</td>
<td>Malignant neoplasm of prostate</td>
</tr>
<tr>
<td>Other HCPCS codes related to the CPB:</td>
<td></td>
</tr>
<tr>
<td>Olaparib (Lynparza), Talazoparib (Talzenna), Rucaparib (Rubraca), Niraparib (Zejula) - no specific code:</td>
<td></td>
</tr>
<tr>
<td>J9043</td>
<td>Injection, cabazitaxel, 1 mg</td>
</tr>
<tr>
<td>J9045</td>
<td>Injection, carboplatin, 50 mg</td>
</tr>
<tr>
<td>J9060</td>
<td>Injection, cisplatin, powder or solution, 10 mg</td>
</tr>
<tr>
<td>J9171</td>
<td>Injection, docetaxel, 1 mg</td>
</tr>
<tr>
<td>J9263</td>
<td>Injection, oxaliplatin, 0.5 mg</td>
</tr>
<tr>
<td>J9264</td>
<td>Injection, paclitaxel protein-bound particles, 1 mg</td>
</tr>
<tr>
<td>J9267</td>
<td>Injection, paclitaxel, 1 mg</td>
</tr>
<tr>
<td>MyChoice CDx:</td>
<td></td>
</tr>
<tr>
<td>CPT codes covered if selection criteria are met:</td>
<td></td>
</tr>
<tr>
<td>0172U</td>
<td>Oncology (solid tumor as indicated by the label), somatic mutation analysis of BRCA1 (BRCA1, DNA repair associated), BRCA2 (BRCA2, DNA repair associated) and analysis of homologous recombination deficiency pathways, DNA, formalin-fixed paraffin-embedded tissue, algorithm quantifying tumor genomic instability score</td>
</tr>
<tr>
<td>BRAF and NRAS mutations (e.g., cobas KRAS Mutation Test; therascreen KRAS RGQ PCR Kit, Praxis Extened RAS Panel):</td>
<td></td>
</tr>
<tr>
<td>CPT codes covered if selection criteria are met:</td>
<td>BRAF (B-Raf proto-oncogene, serine/threonine kinase) (eg, colon cancer,</td>
</tr>
<tr>
<td>81210</td>
<td>BRAF (B-Raf proto-oncogene, serine/threonine kinase) (eg, colon cancer,</td>
</tr>
</tbody>
</table>
### Table: CPT Codes / HCPCS Codes / ICD-10 Codes

*Information in the [brackets] below has been added for clarification purposes. Codes requiring a 7th character are represented by "+":*

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<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>81311</td>
<td>melanoma), gene analysis, V600 variant(s)</td>
</tr>
<tr>
<td></td>
<td>NRAS (neuroblastoma RAS viral [v-ras] oncogene homolog) (eg, colorectal carcinoma), gene analysis, variants in exon 2 (eg, codons 12 and 13) and exon 3 (eg, codon 61)</td>
</tr>
</tbody>
</table>

Other HCPCS codes related to the CPB:

- **J9055** Injection, cetuximab, 10 mg
- **J9303** Injection, panitumumab, 10 mg

ICD-10 codes covered if selection criteria are met:

- **C18.0 - C20** Malignant neoplasm of colon, rectosigmoid junction and rectum

**Epidermal growth factor receptor (EGFR) T790 mutation; exon 19 deletions or exon 21 (L858R) substitution mutations (e.g., cobas EGFR Mutation Test, therascreen EGFR RGQ PCR Kit):**

- **CPT codes covered if selection criteria are met:**
  - **81235** EGFR (epidermal growth factor receptor) (eg, non-small cell lung cancer) gene analysis, common variants (eg, exon 19 LREA deletion, L858R, T790M, G719A, G719S, L861Q)

Other HCPCS codes related to the CPB:

- **J8565** Gefitinib, oral, 250 mg

ICD-10 codes covered if selection criteria are met:

- **C34.00 - C34.92** Malignant neoplasm of bronchus and lung [non-small cell lung carcinoma]

**PD-L1 expression (e.g., PD-L1 IHC 22C3 pharmDx, Ventana PD-L1 (SP263) Assay):**

- **CPT codes covered if selection criteria are met:**
  - **88360** Morphometric analysis, tumor immunohistochemistry (eg, Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, per specimen, each single antibody stain procedure; manual
  - **88361** using computer-assisted technology

Other HCPCS codes related to the CPB:

- **J9022** Injection, atezolizumab, 10 mg
- **J9271** Injection, pembrolizumab, 1 mg

ICD-10 codes covered if selection criteria are met:

- **C34.00 -** Malignant neoplasm of bronchus and lung [non-small cell lung carcinoma]
Table: CPT Codes / HCPCS Codes / ICD-10 Codes

Information in the [brackets] below has been added for clarification purposes. Codes requiring a 7th character are represented by "+";

<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>C34.92</td>
<td></td>
</tr>
<tr>
<td>C50.011 - C50.929</td>
<td>Malignant neoplasm of breast</td>
</tr>
<tr>
<td>C65.1 - C65.9</td>
<td>Malignant neoplasm of renal pelvis [urothelial carcinoma]</td>
</tr>
<tr>
<td>C66.1 - C66.9</td>
<td>Malignant neoplasm of ureter [urothelial carcinoma]</td>
</tr>
<tr>
<td>C67.0 - C67.9</td>
<td>Malignant neoplasm of bladder, unspecified [urothelial carcinoma]</td>
</tr>
<tr>
<td>C68.0 - C68.9</td>
<td>Malignant neoplasm of other and unspecified urinary organs [urothelial carcinoma]</td>
</tr>
</tbody>
</table>

Platelet-derived growth factor receptor-beta (PDGFRß) gene rearrangements (e.g., PDGFRB FISH):

CPT codes covered if selection criteria are met:

88271    Molecular cytogenetics; DNA probe, each (eg, FISH)
88275    interphase in situ hybridization, analyze 100-300 cells

Other HCPCS codes related to the CPB:

S0088    Imatinib, 100 mg

ICD-10 codes covered if selection criteria are met:

C93.10 - C93.12 | Chronic myelomonocytic leukemia
D46.0 - D46.9 | Myelodysplastic syndromes
D47.1 | Chronic myeloproliferative disease

C-KIT testing/ KIT D816V Mutation Detection by PCR:

CPT codes covered if selection criteria are met:

81272 | KIT (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) (eg, gastrointestinal stromal tumor [GIST], acute myeloid leukemia, melanoma), gene analysis, targeted sequence analysis (eg, exons 8, 11, 13, 17, 18)
81273 | KIT (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) (eg, mastocytosis), gene analysis, D816 variant(s)

Other HCPCS codes related to the CPB:

S0088 | Imatinib, 100 mg

ICD-10 codes covered if selection criteria are met:

C49.A0 - C49.A9 | Gastrointestinal stromal tumors
<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>C96.21</td>
<td>Aggressive systemic mastocytosis</td>
</tr>
<tr>
<td></td>
<td><strong>FTL3 mutation assay (e.g., LeukoStrat CDx FLT Mutation Assay):</strong></td>
</tr>
<tr>
<td></td>
<td>CPT codes covered if selection criteria are met:</td>
</tr>
<tr>
<td>0023U</td>
<td>Oncology (acute myelogenous leukemia), DNA, genotyping of internal tandem</td>
</tr>
<tr>
<td></td>
<td>duplication, p.D835, p.I836, using mononuclear cells, reported as detection or</td>
</tr>
<tr>
<td></td>
<td>non-detection of FLT3 mutation and indication for or against the use of</td>
</tr>
<tr>
<td></td>
<td>midostaurin</td>
</tr>
<tr>
<td>81245</td>
<td>FLT3 (fms-related tyrosine kinase 3) (eg, acute nyeloid leukemia), gene analysis;</td>
</tr>
<tr>
<td></td>
<td>internal tandem duplication (ITD) variants (ie, exons 14, 15)</td>
</tr>
<tr>
<td>81246</td>
<td>tyrosine kinase domain (TKD) variants (eg D835, I836)</td>
</tr>
<tr>
<td></td>
<td><strong>Other HCPCS codes related to the CPB:</strong></td>
</tr>
<tr>
<td></td>
<td>Midostaurin (Rydapt), Gilternib (Xospata), Sorafenib (Nexavar) – no specific</td>
</tr>
<tr>
<td></td>
<td>code:</td>
</tr>
<tr>
<td></td>
<td>ICD-10 codes covered if selection criteria are met:</td>
</tr>
<tr>
<td>C92.00 -</td>
<td>Myeloid leukemia</td>
</tr>
<tr>
<td>C92.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Isocitrate dehydrogenase-1 (IDH1), Isocitrate dehydrogenase-2 (IDH2) mutations:</strong></td>
</tr>
<tr>
<td></td>
<td>CPT codes covered if selection criteria are met:</td>
</tr>
<tr>
<td>81120</td>
<td>IDH1 (isocitrate dehydrogenase 1 [NADP+], soluble) (eg, glioma), common</td>
</tr>
<tr>
<td></td>
<td>variants (eg, R132H, R132C)</td>
</tr>
<tr>
<td>81121</td>
<td>IDH2 (isocitrate dehydrogenase 2 [NADP+], mitochondrial) (eg, glioma), common</td>
</tr>
<tr>
<td></td>
<td>variants (eg, R140W, R172M)</td>
</tr>
<tr>
<td></td>
<td><strong>Other HCPCS codes related to the CPB:</strong></td>
</tr>
<tr>
<td></td>
<td>Enasidenib mesylate (Idhifa), Ivosidenib (Tibsovo) - no specific code:</td>
</tr>
<tr>
<td></td>
<td>ICD-10 codes covered if selection criteria are met:</td>
</tr>
<tr>
<td>C92.00 -</td>
<td>Myeloid leukemia</td>
</tr>
<tr>
<td>C92.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Del(17p)/TP53 mutation (e.g., Vysis CLL FISH Probe Kit):</strong></td>
</tr>
<tr>
<td></td>
<td>CPT codes covered if selection criteria are met:</td>
</tr>
<tr>
<td></td>
<td>Del(17p)/TP53 mutation (e.g., Vysis CLL FISH Probe Kit) - no specific code:</td>
</tr>
<tr>
<td></td>
<td>ICD-10 codes covered if selection criteria are met:</td>
</tr>
<tr>
<td>• C91.</td>
<td>Chronic lymphoid leukemia [chronic lymphocytic]</td>
</tr>
<tr>
<td>10 -</td>
<td>C91</td>
</tr>
<tr>
<td>Code</td>
<td>Code Description</td>
</tr>
<tr>
<td>-------</td>
<td>----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>.12,</td>
<td></td>
</tr>
<tr>
<td>• C91</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td></td>
</tr>
<tr>
<td>.91</td>
<td></td>
</tr>
</tbody>
</table>

Other HCPCS codes related to the CPB:

*Venetoclax (Venclexta) - no specific code:*

*BCR/ABL mutations (e.g., MRDx BCR-ABL Test):*

CPT codes covered if selection criteria are met:

*BCR/ABL mutations (e.g., MRDx BCR-ABL Test) - no specific code:*

ICD-10 codes covered if selection criteria are met:

<table>
<thead>
<tr>
<th>C92.10</th>
<th>Myeloid leukemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>C92.92</td>
<td></td>
</tr>
</tbody>
</table>

Other HCPCS codes related to the CPB:

*Nilotinib (Tasigna) – no specific code:*

*FGFR2 and FGFR3 mutation testing:*

CPT codes covered if selection criteria are met:

*FGFR2 mutation testing - no specific code:*

| 0154U  | FGFR3 (fibroblast growth factor receptor 3) gene analysis (ie, p.R248C [c.742C>T], p.S249C [c.746C>G], p.G370C [c.1108G>T], p.Y373C [c.1118A>G], FGFR3-TACC3v1, and FGFR3-TACC3v3) |

Other HCPCS codes related to the CPB:

*Erdafitinib (Balversa), Pemigatinib (Pemazyre) - no specific code:*

ICD-10 codes covered if selection criteria are met:

<table>
<thead>
<tr>
<th>C22.1</th>
<th>Intrahepatic bile duct carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>C65.1</td>
<td>Malignant neoplasm of renal pelvis [urothelial carcinoma]</td>
</tr>
<tr>
<td>C65.9</td>
<td></td>
</tr>
<tr>
<td>C66.1</td>
<td>Malignant neoplasm of ureter [urothelial carcinoma]</td>
</tr>
<tr>
<td>C66.9</td>
<td></td>
</tr>
<tr>
<td>C67.0</td>
<td>Malignant neoplasm of bladder, unspecified [urothelial carcinoma]</td>
</tr>
<tr>
<td>C67.9</td>
<td></td>
</tr>
<tr>
<td>C68.0</td>
<td>Malignant neoplasm of other and unspecified urinary organs [urothelial carcinoma]</td>
</tr>
<tr>
<td>C68.9</td>
<td></td>
</tr>
</tbody>
</table>

*PIK3CA mutation testing:*

CPT codes covered if selection criteria are met:
Table: CPT Codes / HCPCS Codes / ICD-10 Codes
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<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0177U</td>
<td>Oncology (breast cancer), DNA, PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha) gene analysis of 11 gene variants utilizing plasma, reported as PIK3CA gene mutation status</td>
</tr>
<tr>
<td>81309</td>
<td>PIK3CA (phosphatidylinositol-4, 5-biphosphate 3-kinase, catalytic subunit alpha) (eg, colorectal and breast cancer) gene analysis, targeted sequence analysis (eg, exons 7, 9, 20)</td>
</tr>
</tbody>
</table>

CPT codes not covered for indications listed in the CPB:

<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0037U</td>
<td>Targeted genomic sequence analysis, solid organ neoplasm, DNA analysis of 324 genes, interrogation for sequence variants, gene copy number amplifications, gene rearrangements, microsatellite instability and tumor mutational burden</td>
</tr>
</tbody>
</table>

Other HCPCS codes related to the CPB:

Alpelizib (Piqray) - no specific code:

ICD-10 codes covered if selection criteria are met:

| C50.011 - C50.929 | Malignant neoplasm of breast |

Mesenchymal-epithelial transition (MET) testing:

CPT codes covered if selection criteria are met:

| 0037U | Targeted genomic sequence analysis, solid organ neoplasm, DNA analysis of 324 genes, interrogation for sequence variants, gene copy number amplifications, gene rearrangements, microsatellite instability and tumor mutational burden |

Other HCPCS codes related to the CPB:

Capmatinib (Tabrecta) - no specific code:

ICD-10 codes covered if selection criteria are met:

| C34.00 - C34.92 | Malignant neoplasm of bronchus and lung [non-small cell lung cancer] |

Measurement of microsatellite instability and mismatch repair:

CPT codes covered if selection criteria are met:

| 81301 | Microsatellite instability analysis (eg, hereditary non-polyposis colorectal |
### Table: CPT Codes / HCPCS Codes / ICD-10 Codes

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<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cancer, Lynch syndrome) of markers for mismatch repair deficiency (eg, BAT25, BAT26), includes comparison of neoplastic and normal tissue, if performed</td>
</tr>
<tr>
<td>88341</td>
<td>Immunohistochemistry or immunocytochemistry, per specimen; each additional single antibody stain procedure (List separately in addition to code for primary procedure)</td>
</tr>
<tr>
<td>88342</td>
<td>Immunohistochemistry or immunocytochemistry, per specimen; initial single antibody stain procedure [mismatch repair]</td>
</tr>
</tbody>
</table>

**Other HCPCS codes related to the CPB:**

- **J9271** Injection, pembrolizumab, 1 mg

**ICD-10 codes covered if selection criteria are met:**

- **C00.0 - C14.8** Malignant neoplasm of lip, oral cavity and pharynx
- **C15.3 - C26.9** Malignant neoplasm of digestive organs
- **C30.0 - C39.9** Malignant neoplasm of respiratory and intrathoracic organs
- **C40.00 - C41.9** Malignant neoplasm of bone and articular cartilage
- **C49.0 - C49.9** Malignant neoplasm of other connective and soft tissue
- **C7A.00 - C7A.8** Malignant neuroendocrine tumors

**Extended RAS Panel - no specific code:**

*Anaplastic lymphoma kinase (ALK) fusion gene testing, (Vysis ALK Break Apart FISH Probe Kit; Ventana ALK (D5F3) CDx Assay):*

No specific code

**Other HCPCS codes related to the CPB:**

- **Alectinib (Alecensa) - no specific code:**
  - **C34.00** Malignant neoplasm of bronchus and lung [non-small cell lung carcinoma]

**Genotyping for IL28B polymorphism:**

**ICD-10 codes not covered for indications listed in the CPB:**

- **B19.20** Unspecified viral hepatitis C without hepatic coma

**rs3798220 allele testing:**

No specific code
<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICD-10 codes not covered for indications listed in the CPB:</td>
<td></td>
</tr>
<tr>
<td>Z79.82</td>
<td>Long-term (current) use of aspirin</td>
</tr>
<tr>
<td></td>
<td>No specific code</td>
</tr>
<tr>
<td>ICD-10 covered for indications listed in the CPB:</td>
<td></td>
</tr>
<tr>
<td>E84.0 - E84.9</td>
<td>Cystic fibrosis [who are being considered for treatment with ivacaftor (Kalydeco)]</td>
</tr>
<tr>
<td></td>
<td><em>F508del mutation in the CFTR testing:</em></td>
</tr>
<tr>
<td>CPT codes covered if selection criteria are met:</td>
<td></td>
</tr>
<tr>
<td>81222</td>
<td>CFTR (cystic fibrosis transmembrane conductance regulator) (eg, cystic fibrosis) gene analysis; duplication/deletion variants</td>
</tr>
<tr>
<td>ICD-10 codes covered for indications listed in the CPB:</td>
<td></td>
</tr>
<tr>
<td>E84.0-E84.9</td>
<td>Cystic fibrosis [who are being considered for treatment with lumacaftor/ivacaftor (Orkambi)]</td>
</tr>
<tr>
<td></td>
<td><em>GeneSightRx testing (GeneSight ADHD, GeneSight Analgesic, GeneSight MTHFR and GeneSight Psychotropic):</em></td>
</tr>
<tr>
<td></td>
<td>No specific code</td>
</tr>
<tr>
<td>ICD-10 codes not covered for indications listed in the CPB:</td>
<td></td>
</tr>
<tr>
<td>F30.10 - F39</td>
<td>Mood [affective] disorders</td>
</tr>
<tr>
<td></td>
<td><em>Platelet reactivity/function testing (VerifyNow P2Y12 Assay):</em></td>
</tr>
<tr>
<td></td>
<td>No specific code</td>
</tr>
<tr>
<td>ICD-10 codes not covered for indications listed in the CPB:</td>
<td></td>
</tr>
<tr>
<td>Z98.61</td>
<td>Coronary angioplasty status</td>
</tr>
<tr>
<td>OnDose lab test:</td>
<td></td>
</tr>
<tr>
<td>HCPCS codes not covered for indications listed in the CPB:</td>
<td></td>
</tr>
<tr>
<td>S3722</td>
<td>Dose optimization by area under the curve (AUC) analysis, for infusional 5- Fluorouracil</td>
</tr>
<tr>
<td>MGMT (O(6)- methylguanine-DNA methyltransferase) gene methylation Assay:</td>
<td></td>
</tr>
<tr>
<td>CPT codes covered if selection criteria are met:</td>
<td></td>
</tr>
<tr>
<td>81287</td>
<td>MGMT (O-6-methylguanine-DNA methyltransferase) (eg, glioblastoma multiforme), methylation analysis</td>
</tr>
<tr>
<td>Other HCPCS codes related to the CPB:</td>
<td></td>
</tr>
<tr>
<td>J8700</td>
<td>Temozolomide, oral, 5 mg</td>
</tr>
</tbody>
</table>
### Table: CPT Codes / HCPCS Codes / ICD-10 Codes

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<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>J9328</td>
<td>Injection, temozolomide, 1 mg</td>
</tr>
</tbody>
</table>

**ICD-10 covered for indications listed in the CPB:**

- **C71.0 - C71.9**: Malignant neoplasm of brain [glioblastoma]
  
**Genecept Assay:**

**CPT codes not covered for indications listed in the CPB:**

- **0173U**: Psychiatry (ie, depression, anxiety), genomic analysis panel, includes variant analysis of 14 genes
- **0175U**: Psychiatry (eg, depression, anxiety), genomic analysis panel, variant analysis of 15 genes

**ICD-10 codes not covered for indications listed in the CPB (not all inclusive):**

- **F20.0 - F20.9**: Schizophrenia
- **F30.10 - F39**: Mood [affective] disorders
- **F32.0 - F32.9**: Major depressive disorders
- **F34.0 - F34.9**: Persistent mood [affective] disorders
- **F40.00 - F48.9**: Anxiety, dissociative, stress-related, somatoform and other nonpsychotic mental disorders
- **F90.1 - F90.9**: Attention-deficit hyperactivity disorder

**Beta adrenergic receptor genotyping:**

**CPT codes not covered for indications listed in the CPB:**

- **81401**: VKORC1 (vitamin K epoxide reductase complex, subunit 1) (eg, warfarin metabolism), gene analysis, common variant(s) (eg, -1639G>A, c.173+1000C>T)

**ICD-10 codes not covered for indications listed in the CPB:**

- **J45.20 - J45.998**: Asthma [treatment resistant]

**Methotrexate polyglutamates (Avise PG test):**

- No specific code

**Other HCPCS codes related to the CPB:**

- **J8610**: Methotrexate; oral, 2.5 mg
- **J9250**: Methotrexate sodium, 5 mg
- **J9260**: Methotrexate sodium, 50 mg

**ICD-10 codes not covered for indications listed in the CPB (not all inclusive):**
<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>M05.00 - M14.89</td>
<td>Inflammatory polyarthropathies</td>
</tr>
</tbody>
</table>

**Millennium PGT:**

No specific code

**ICD-10 codes not covered for indications listed in the CPB (not all inclusive):**

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>G89.21 - G89.29</td>
<td>Chronic pain, not elsewhere classified</td>
</tr>
</tbody>
</table>

**PersonaGene Genetic Panels:**

**CPT codes not covered for indications listed in the CPB:**

<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>81225</td>
<td>CYP2C19 (cytochrome P450, family 2, subfamily C, polypeptide 19) (eg, drug metabolism), gene analysis, common variants (eg, '2, *3, '4, '8, '17)</td>
</tr>
<tr>
<td>81240</td>
<td>F2 (prothrombin, coagulation factor II) (eg, hereditary hypercoagulability) gene analysis, 20210G&gt;A variant</td>
</tr>
<tr>
<td>81241</td>
<td>F5 (coagulation factor V) (eg, hereditary hypercoagulability) gene analysis, Leiden variant</td>
</tr>
<tr>
<td>81291</td>
<td>MTHFR (5,10-methylenetetrahydrofolate reductase) (eg, hereditary hypercoagulability) gene analysis, common variants (eg, 677T, 1298C)</td>
</tr>
<tr>
<td>81355</td>
<td>VKORC1 (vitamin K epoxide reductase complex, subunit 1) (eg, warfarin metabolism), gene analysis, common variant(s) (eg, -1639G&gt;A, c.173+1000C&gt;T)</td>
</tr>
<tr>
<td>81401</td>
<td>VKORC1 (vitamin K epoxide reductase complex, subunit 1) (eg, warfarin metabolism), gene analysis, common variant(s) (eg, -1639G&gt;A, c.173+1000C&gt;T)</td>
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**ICD-10 codes not covered for indications listed in the CPB (not all inclusive):**

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<tbody>
<tr>
<td>Z51.81</td>
<td>Encounter for therapeutic drug level monitoring</td>
</tr>
</tbody>
</table>

**HLA-B 58:01:**

**CPT codes covered if selection criteria are met:**

No specific code

**Aegis Drug-Drug Interaction Test:**
<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0006U</td>
<td>Prescription drug monitoring, 120 or more drugs and substances, definitive tandem mass spectrometry with chromatography, urine, qualitative report of presence (including quantitative levels, when detected) or absence of each drug or substance with description and severity of potential interactions, with identified substances, per date of service</td>
</tr>
<tr>
<td>0015U</td>
<td>Drug metabolism (adverse drug reactions), DNA, 22 drug metabolism and transporter genes, real-time PCR, blood or buccal swab, genotype and metabolizer status for therapeutic decision support</td>
</tr>
<tr>
<td>81283</td>
<td>IFNL3 (interferon, lambda 3) (eg, drug response), gene analysis, rs12979860 variant</td>
</tr>
<tr>
<td>F40.00 - F48.9</td>
<td>Anxiety, dissociative, stress-related, somatoform and other nonpsychotic mental disorders</td>
</tr>
<tr>
<td></td>
<td>• K21.0, K21.9 Gastro-esophageal reflux disease with or without esophagitis</td>
</tr>
<tr>
<td>0025U</td>
<td>Tenofovir, by liquid chromatography with tandem mass spectrometry (LC-MS/MS), urine, quantitative</td>
</tr>
<tr>
<td>Z20.6</td>
<td>Contact with and (suspected) exposure to human immunodeficiency virus [HIV]</td>
</tr>
<tr>
<td>Z20.828</td>
<td>Contact with and (suspected) exposure to other viral communicable diseases</td>
</tr>
<tr>
<td>Code</td>
<td>Code Description</td>
</tr>
<tr>
<td>-----------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>0078U</td>
<td>Pain management (opioid-use disorder) genotyping panel, 16 common variants (ie, ABCB1, COMT, DAT1, DBH, DOR, DRD1, DRD2, DRD4, GABA, GAL, HTR2A, HTTLPR, MTHFR, MUOR, OPRK1, OPRM1), buccal swab or other germline tissue sample, algorithm reported as positive or negative risk of opioid-use disorder</td>
</tr>
<tr>
<td></td>
<td><strong>ComplyRX urine test:</strong></td>
</tr>
<tr>
<td>0093U</td>
<td>Prescription drug monitoring, evaluation of 65 common drugs by LC-MS/MS, urine, each drug reported detected or not detected</td>
</tr>
<tr>
<td>0110U</td>
<td>Prescription drug monitoring, one or more oral oncology drug(s) and substances, definitive tandem mass spectrometry with chromatography, serum or plasma from capillary blood or venous blood, quantitative report with steady-state range for the prescribed drug(s) when detected</td>
</tr>
<tr>
<td>0116U</td>
<td>Prescription drug monitoring, enzyme immunoassay of 35 or more drugs confirmed with LC-MS/MS, oral fluid, algorithm results reported as a patient-compliance measurement with risk of drug to drug interactions for prescribed medications</td>
</tr>
<tr>
<td>Z51.81</td>
<td>Encounter for therapeutic drug level monitoring</td>
</tr>
</tbody>
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**ICD-10 codes not covered for indications listed in the CPB:**

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<tr>
<td>Z51.81</td>
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</tr>
</tbody>
</table>

**Experimental and Investigation Treatment - no specific codes:**

A1555G, Amerigene PGT pharmacogenetic testing panel, Proove Profiles (including Proove Opioid Risk Panel, Proove Opioid Response Profile, Proove Non-opioid Response Profile, Proove Opioid-Induced Side Effects Profile, Proove NSAID Risk Profile, Proove Fibromyalgia Profile, Proove Epidural with Fentanyl Response Profile, Proove MAT (Medically Assisted Treatment) Response Profile, Proove TMD Profile, Proove Psychiatric Risk and Response Profile, Proove Addiction Profile, Proove Narcotic Risk Panel, Proove Drug Metabolism Panel, rxSEEK Epilepsy Drug Metabolism Test (Courtagen Life Sciences)

**The above policy is based on the following references:**

**General References**


Genotyping for Cytochrome P450 Polymorphisms


34. Ndewga, S. Pharmacogenomics and warfarin therapy. Issues in Emerging Health Technologies Issue 104. Ottawa, ON: Canadian Agency for Drugs and Technologies in Health (CADTH); 2007.
45. Valeant Pharmaceuticals North America, LLC. Xenazine (tetrabenazine) tablets, for oral use. Prescribing Information. Laval QC: Valeant; revised September 2018.

**VKORC1 Assay**


**UGT1A1 Molecular Assay**


**Genotyping for HLA-B*1502**


**HLA-B*5701 Screening**


**Genotyping for Apolipoprotein E (Apo E)**


Genotyping for Methylenetetrahydrofolate Reductase (MTHFR)


Measurement of Thromboxane Metabolites in Urine


**Genetic Testing for rs3798220 Allele (LPA-Aspirin Check)**


**AUC-Targeted 5-Fluorouracil Dosing and Pharmacogenetic Testing for 5-FU Toxicity**

1. BlueCross BlueShield Association (BCBSA), Technology Evaluation Center (TEC). Special report: Laboratory testing to allow area under the curve (AUC) –targeted 5-fluorouracil dosing for patients administered chemotherapy for cancer, TEC Assessment Program. Chicago, IL: BCBSA; 2010;24(10).
2. BlueCross BlueShield Association (BCBSA), Technology Evaluation Center (TEC). Pharmacogenetic testing to predict serious toxicity from 5-fluorouracil (5-FU) for patients administered 5-FU-based chemotherapy for cancer. TEC Assessments in Press. Chicago, IL: BCBSA; January 2010.

**CYP2D6 Polymorphism and Alzheimer’s Disease**


**The Vysis ALK Break Apart FISH Probe Kit**


**GeneSightRx Testing**


**GeneCept Testing**


**IDgenetix Testing**


CNSDose Testing


ABCB1 Gene Testing


Platelet Reactivity Testing (VerifyNow P2Y12 Assay)


**Beta Adrenergic Receptor Genotyping (ADRB2) for Treatment-Resistant Asthma**

11. Kazani S et al. The role of pharmacogenomics in improving the management of asthma. Journal of Allergy and Clinical Immunology, Volume 125, Issue 2 2010.

**MGMT Assay for Glioblastoma**


**Mutation Testing in the BRAF Gene**


**Millennium PGT**

PROOVE Opioid Risk Test

1. Piller C. Proove Biosciences, which sold dubious DNA tests to predict addiction risk, sells off assets as CEO departs amid criminal probe. STAT, August 31, 2017.

BRACAnalysis CDx


Thiopurine S-Methyltransferase (TPMT)


FLT3 Mutation for Midostaurin (Rydapt)


Aegis Drug-Drug Interaction Test

Proprietary

OneOme RightMed Pharmacogenomic Test


CYP2C19 Polymorphisms Testing for Individuals on Fluoxetine


PGxOnePlus


Genotyping of Interferon-Lambda 3 (IFNL3) for Prediction of Virological Response to Pegylated-Interferon-Alpha and Ribavirin Combination Therapy


UrSure Tenofovir Quantification Test

**INFINITI Neural Response Panel**


**Snapshot Oral Fluid Compliance**


**OncolyticAssuranceRX**


**Mutation Testing for microsatellite instability (MSI-H) and mismatch repair deficiency (DMMR)**


**EZH2 Testing for tazemetostat (Tazverik) therapy**


**FGFR Testing for Pemigatinib therapy**

Amendment to
Aetna Clinical Policy Bulletin Number: 0715
Pharmacogenetic and Pharmacodynamic Testing


The Pennsylvania Medical Assistance Program also considers an FDA-approved test for the anaplastic lymphoma kinase (ALK) fusion gene (e.g., the Vysis ALK Break Apart FISH Probe Kit; Ventana ALK (D5F3) CDx Assay) medically necessary for persons who are considering alectinib (Alecensa) for the treatment of non-small cell lung cancer (NSCLC).

The Pennsylvania Medical Assistance Program also considers the following tests to medically necessary:

- c-KIT expression, c-KITD816V mutation in persons with GIST or ATS considering imatinib therapy
- Chemokine C-C motif receptor 5 in persons considering therapy with maraviroc
- Deletion of chromosome 5q in persons with myelodysplastic syndrome considering therapy with lenalidomide
- G6PD deficiency in persons considering therapy with Rasburicase, dapsone, primaquine or chloroquine
- HER2 overexpression in persons considering therapy with Trastuzumab, pertuzumab or lapatinib
- Philadelphia chromosome positivity in persons with ALL considering therapy with dasatinib.

The Pennsylvania Medical Assistance Program also considers testing for biomarkers as specified in NCCN clinical practice guidelines or FDA approved package labeling for indication and usage, or dose and administration to be medically necessary.

revised 10/09/2020