Supplementary Online Content

Povsic TJ, Ohman EM, Roe MT, et al. P2Y12 inhibitor switching in response to routine notification of CYP2C19 clopidogrel metabolizer status following acute coronary syndromes. *JAMA Cardiol*. Published online May 22, 2019. doi:10.1001/jamacardio.2019.1510

This supplementary material has been provided by the authors to give readers additional information about their work.

© 2019 American Medical Association. All rights reserved.

Methods

Investigator Training

All investigators were informed that pharmacogenomics sample for assessment of CYP2C19 metabolizer status was required and would be obtained from all participants. Training at investigator's meetings and at sessions with individual investigators at site meetings informed them that the results of genotyping would be provided for all subjects regardless of P2Y12 inhibitor choice (clopidogrel or ticagrelor) and any prior CYP2C19 genotype testing. Investigators were mandated to inform subjects of the results. It was our intent to NOT influence decision making and to allow investigators to use this information at their discretion, thus, we intentionally provided no specific instructions or guidance as to how to interpret or utilize these results; however, investigators were required to prespecify what actions they expected to take with regard to P2Y12 inhibitor choice once this data was provided.

Information regarding interpretation of genotyping was provided (See additional supplemental material).

In the CRF, the principal investigators were asked to specify one of the following prior to randomization:

1. This patient is treated with clopidogrel, and I will not change P2Y12 inhibitor based upon the genotyping results.
2. This patient is treated with clopidogrel, and I will switch to ticagrelor if a reduced function CYP2C19 metabolism allele is identified.
3. This patient is treated with ticagrelor, and I will not change P2Y12 inhibitor based upon the genotyping results.
4. This patient is treated with ticagrelor, and I will switch to clopidogrel if the normal CYP2C19 metabolism allele is identified.

Statistical methods

Patterns of P2Y12 inhibitor switching and reasons for switching, as recorded in the case report form, were evaluated by randomized treatment assignment (aspirin vs. rivaroxaban) and within the overall study population. Time from randomization to date of the prescribed P2Y12 inhibitor switching (as recorded by the investigators) was evaluated by reasons for switching.

Investigators were required to prestipulate whether they would switch the choice of the initial P2Y12 inhibitor based on the reported CYP2C19 metabolizer status. Actual P2Y12 inhibitor switching by prestipulated intention was evaluated based on initial P2Y12 inhibitor use (clopidogrel vs. ticagrelor) and within the overall population stratified by CYP2C19 metabolizer status categories.

© 2019 American Medical Association. All rights reserved.

eTable 1. Comparison of patient characteristics: switching versus no switching

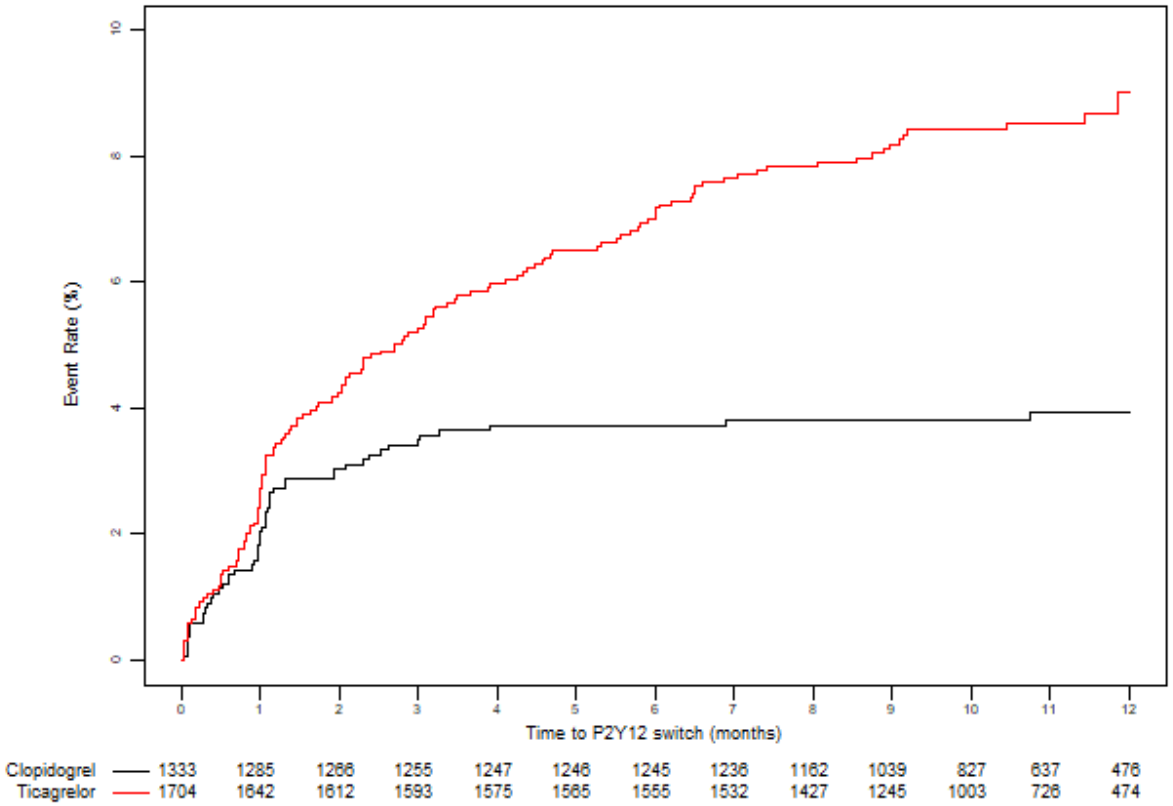
	No Switch (n=2840)	Switch (n=197)	Total (n=3037)
Age, mean (SD), yrs	62.7 (9.0)	64.2 (8.8)	62.8 (9.0)
Male	2119 (74.6%)	156 (79.2%)	2275 (74.9%)
White	2641 (93.0%)	183 (92.9%)	2824 (93.0%)
Hispanic or Latino	354 (12.6%)	21 (11.1%)	375 (12.5%)
Weight, mean (SD), kg	82.6 (16.0)	83.8 (16.9)	82.7 (16.1)
BMI, mean (SD), kg/m^2	28.5 (4.8)	28.3 (4.5)	28.5 (4.8)
Region			
Eastern Europe	715 (25.2%)	32 (16.2%)	747 (24.6%)
Western Europe	561 (19.8%)	57 (28.9%)	618 (20.3%)
Central Europe	919 (32.4%)	47 (23.9%)	966 (31.8%)
North America	232 (8.2%)	33 (16.8%)	265 (8.7%)
South America	274 (9.6%)	19 (9.6%)	293 (9.6%)
Asia and Pacific	139 (4.9%)	9 (4.6%)	148 (4.9%)
Smoking status			
Never	1037 (36.5%)	67 (34.0%)	1104 (36.4%)
Current	941 (33.1%)	56 (28.4%)	997 (32.8%)
Former	862 (30.4%)	74 (37.6%)	936 (30.8%)
Diabetes	856 (30.1%)	50 (25.4%)	906 (29.8%)
Hypertension	2092 (73.7%)	132 (67.0%)	2224 (73.2%)
Hyperlipidemia	1596 (56.2%)	113 (57.4%)	1709 (56.3%)
Prior CABG	111 (3.9%)	15 (7.6%)	126 (4.1%)
Prior PCI	557 (19.6%)	44 (22.3%)	601 (19.8%)
Prior MI	611 (21.5%)	48 (24.4%)	659 (21.7%)
Prior PAD	126 (4.4%)	14 (7.1%)	140 (4.6%)
CrCl, mean (SD) mL/min	90.3 (30.3)	87.8 (30.4)	90.2 (30.3)
Baseline hemoglobin, mean (SD), mg/dL	14.1 (1.6)	14.1 (1.5)	14.1 (1.6)
Qualifying event			
NSTEMI	1141 (40.2%)	82 (41.6%)	1223 (40.3%)
STEMI	1384 (48.7%)	100 (50.8%)	1484 (48.9%)
Unstable angina	315 (11.1%)	15 (7.6%)	330 (10.9%)
Catheterization at qualifying event	2666 (93.9%)	189 (95.9%)	2855 (94.0%)
PCI at qualifying event	2471 (87.0%)	174 (88.3%)	2645 (87.1%)
CYP2C19 metabolizer status			
Ultra-rapid metabolizer	982 (34.8%)	57 (28.9%)	1039 (34.4%)
Extensive metabolizer	1079 (38.3%)	61 (31.0%)	1140 (37.8%)
Intermediate metabolizer	681 (24.2%)	58 (29.4%)	739 (24.5%)
Reduced metabolizer	77 (2.7%)	21 (10.7%)	98 (3.2%)
Randomized treatment			
Aspirin	1413 (49.8%)	105 (53.3%)	1518 (50.0%)
Rivaroxaban	1427 (50.2%)	92 (46.7%)	1519 (50.0%)
Initial P2Y12 inhibitor			
Clopidogrel	1282 (45.1%)	51 (25.9%)	1333 (43.9%)
Ticagrelor	1558 (54.9%)	146 (74.1%)	1704 (56.1%)

Data presented as no. (%), unless otherwise indicated.

BMI indicates body mass index; CABG, coronary artery bypass grafting; CrCl, creatinine clearance; MI, myocardial infarction; NSTEMI, non-ST segment elevation myocardial infarction; PAD, peripheral artery disease; PCI, percutaneous coronary intervention; SD, standard deviation; STEMI, ST segment elevation myocardial infarction.

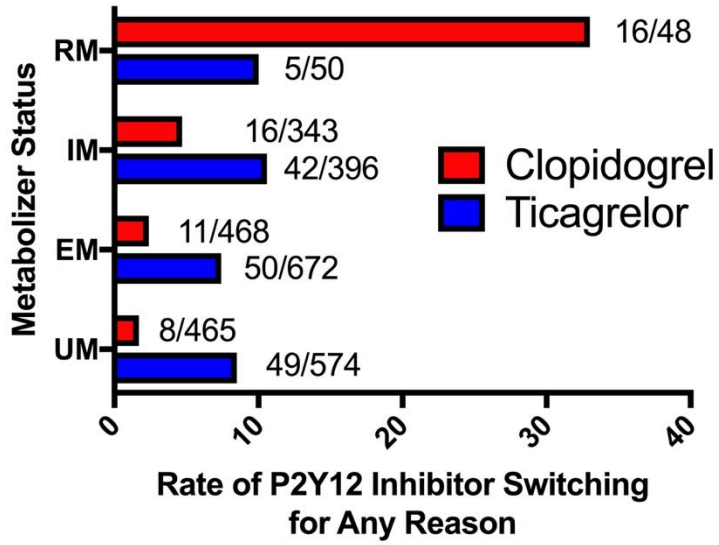
© 2019 American Medical Association. All rights reserved.

eFigure 1. Frequency of P2Y12 inhibitor switching for any reason by initial choice of P2Y12 inhibitor (clopidogrel vs. ticagrelor) over time



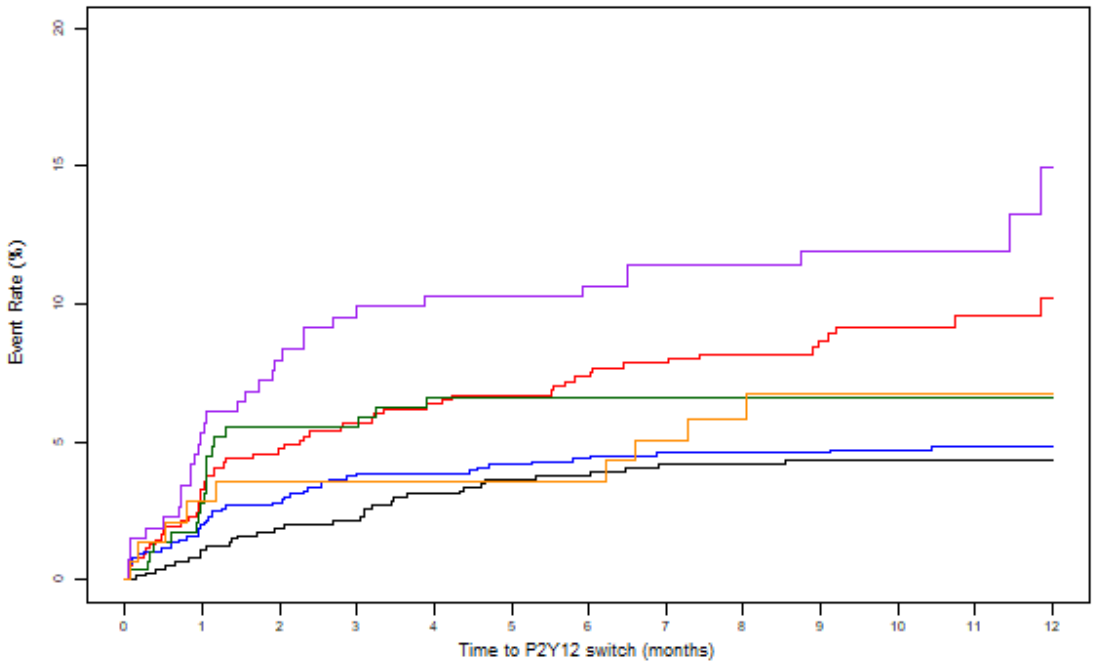
© 2019 American Medical Association. All rights reserved.

eFigure 2. Frequency of overall P2Y12 inhibitor switching for any reason by metabolizer status and initial P2Y12 inhibitor choice



© 2019 American Medical Association. All rights reserved.

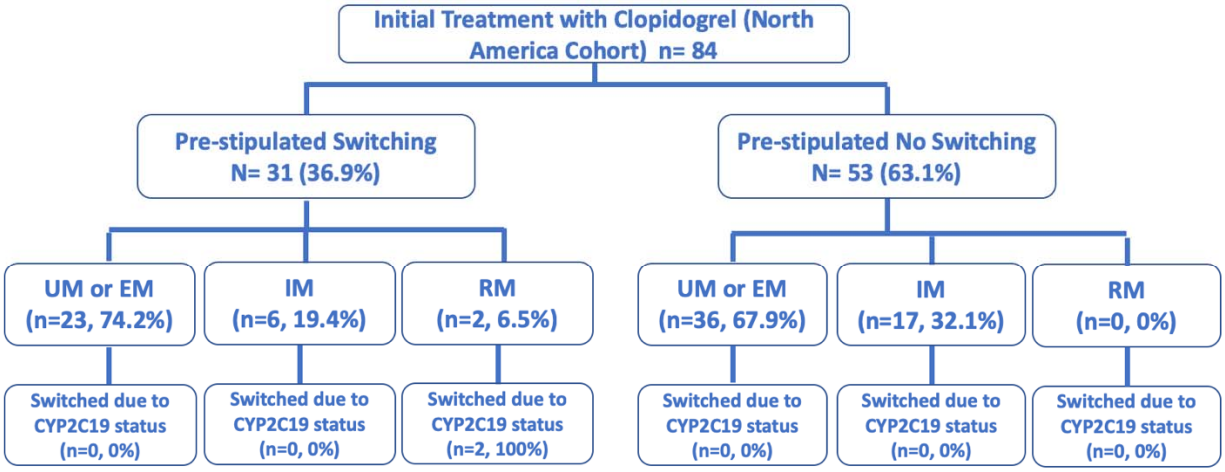
eFigure 3. Time course of P2Y12 inhibitor switching by region



	Eastern Europe	Western Europe	Central Europe	North America	South America	Asia & Pacific	Overall
P2Y12 Switching	32/747 (4.3%)	57/618 (9.2%)	47/966 (4.9%)	33/265 (12.5%)	19/293 (6.5%)	9/148 (6.1%)	197/3037 (6.5%)

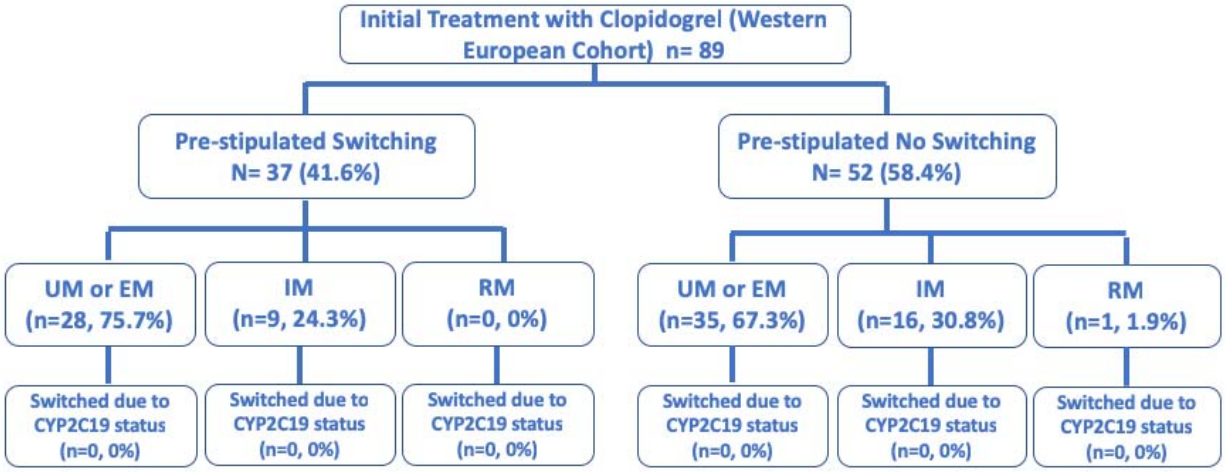
© 2019 American Medical Association. All rights reserved.

eFigure 4A. Flow chart depicting switching due to CYP2C19 status by metabolizer status and pre-stipulated intent in patients initially treated with clopidogrel in North America



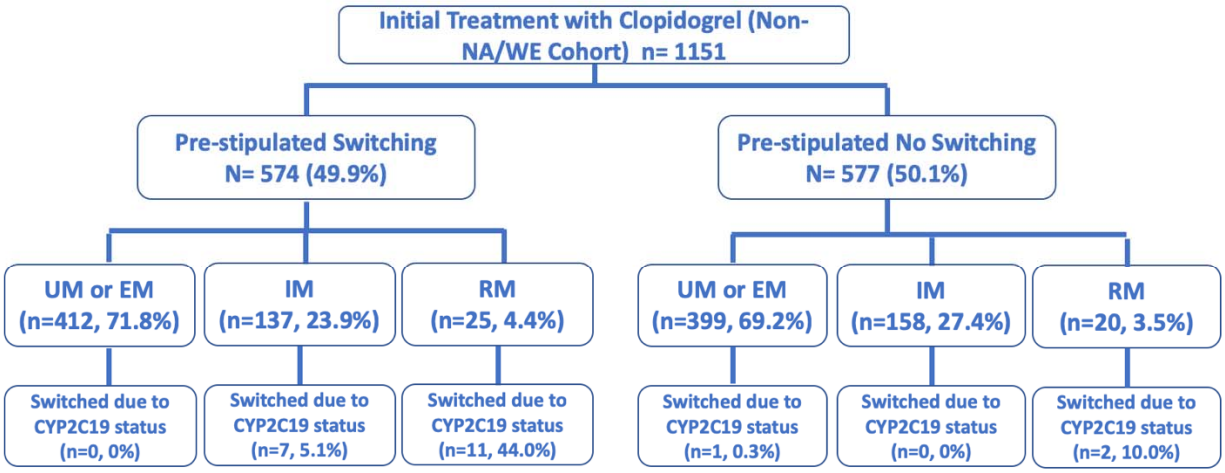
© 2019 American Medical Association. All rights reserved.

eFigure 4B. Flow chart depicting switching due to CYP2C19 status by metabolizer status and pre-stipulated intent in patients initially treated with clopidogrel in Western Europe



© 2019 American Medical Association. All rights reserved.

eFigure 4C. Flow chart depicting switching due to CYP2C19 status by metabolizer status and pre-stipulated intent in patients initially treated with clopidogrel in non-North American or Western European countries



© 2019 American Medical Association. All rights reserved.

CYP2C19 Genotype Interpretation

Assay description

Genetic variants of CYP2C19 are associated with significant enzyme activity variations that alter the rate of drug metabolism, ranging from poor metabolizers to ultra-rapid metabolizers and may cause increased or decreased drug efficacy or adverse drug reactions.
The AutoGenomics INFINITI CYP450 2C19+ Assay is designed to identify 10 genetic variants in the 2C19 gene in human genomic DNA obtained from EDTA-anticoagulated whole blood samples, as described in table 1.
The assay detects the following CYP2C19 alleles: *2, *3, *4, *5, *6, *7, *8, *9, *10, *17. Allele*1 ("wild type") is reported if all other alleles measured by the assay are absent.
The Autogenomics INFINITI CYP2C19+ Assay is a *Research Use Only* (RUO) assay.

CYP2C19 Allele	Polymorphism Recognized by the INFINITI 2C19+ Assay	Enzyme Activity
*1	None	Normal
*2	19154G>A	None
*3	17948G>A	None
*4	1A>G	None
*5	90033C>T	None
*6	12748G>A	None
*7	19294T>A	None
*8	12711T>C	None
*9	12784G>A	Decreased
*10	19153C>T	Decreased
*17	-806C>T	Increased

CYP2C19 allele frequency is dependent on ethnicity. The most common non-functional alleles are *2 and *3
*2 Asians – 30% Caucasians and African Americans – 15%
*3 Asians – 8% Caucasians and African Americans – <1%
Frequency of Poor Metabolizer status (caused by two non-functional CYP2C19 alleles) is as follow:
Caucasians – 4% African Americans – 5% Asians – up to 25%

Metabolizer status

Genetic variants (or alleles) of CYP2C19 are associated with significant phenotypic variations that alter the rate of drug metabolism and may cause increased or decreased drug efficacy or adverse drug reactions. Metabolizer status (Predictive Phenotype) can be assigned based on the CYP2C19 genotype identified by the assay.

Table 2 - Assignment of CYP2C19 Metabolizer Status (Predictive Phenotype) based on genotypes

Predictive Phenotype	Genotypes	Examples of Genotypes
Ultra-rapid metabolizer (UM)	An individual carrying two increased activity alleles (*17) or one functional allele (*1) plus one increased activity allele (*17)	*1/*17, *17/*17
Extensive metabolizer (EM)	An individual carrying two functional (*1) alleles	*1/*1
Intermediate metabolizer (IM)	An individual carrying one functional allele (*1) plus one loss-of-function allele (*2,*3) or one loss-of-function allele (*2,*3) plus one increased activity allele (*17)	*1/*2,*1/*3, *2/*17
Poor metabolizer (PM)	An individual carrying two loss-of-function alleles (*2,*3)	*2/*2,*2/*3, *3/*3

©2010 Covance CLS

© 2019 American Medical Association. All rights reserved.

CYP2C19 Genotype Interpretation

Result Reporting

For each sample, the Genotype and Metabolizer status interpretation are reported. The metabolizer status is reported as one of the following: Extensive Metabolizer (EM - Normal CYP2C19 metabolism), Ultra Metabolizer (UM), Intermediate Metabolizer (IM), Poor Metabolizer (PM)

Table 3 – Metabolizer status Interpretation (Predictive Phenotype) associated with each possible CYP2C19 genotypes reported by this assay

	*1	*2	*3	*4	*5	*6	*7	*8	*9	*10	*17
*1	EM	IM	IM	IM	IM	IM	IM	IM	IM	IM	UM
*2		PM	PM	PM	PM	PM	PM	PM	PM	PM	IM[1]
*3			PM	PM	PM	PM	PM	PM	PM	PM	IM[1]
*4				PM	PM	PM	PM	PM	PM	PM	IM[1]
*5					PM	PM	PM	PM	PM	PM	IM[1]
*6						PM	PM	PM	PM	PM	IM[1]
*7							PM	PM	PM	PM	IM[1]
*8								PM	PM	PM	IM[1]
*9									IM	IM	IM[1]
*10										IM	IM[1]
*17											UM

[1] - The predictive phenotype for these genotypes is provisional classifications. The currently available evidence indicates that the *17 gain-of-function allele is unable to completely compensate for the *2 loss-of-function allele. The currently available data have not been consistently replicated and is therefore a provisional classification (1).

Assay limitations

- CYP2C19 variants other than *2, *3, *4, *5, *6, *7, *8, *9, *10, *17 are not evaluated by this assay.
- The normal enzymatic activity (*1 “wild-type”) status is reported if all other alleles that are measured by the assay are absent. This assay does not interrogate some rare frequency alleles (*11, *12, *13, *14, *15, *16). These variants may or may not be present in the sample. If they are present, they may be erroneously reported as “wild type”.
- The INFINITI CYP2C19+ Assay does not determine whether alleles are present on the same or opposite chromosomes. Phenotypic interpretation of CYP2C19 heterozygous genotypes is based on the assumption that alleles are present on opposite chromosomes.
- Drug metabolism may be affected by non-genetic factors such as drug/drug interactions and conditions that impair renal or hepatic function. Genotype results should be interpreted in the context of the individual clinical situation. Genotype results are not a substitute for therapeutic drug or clinical monitoring.
- Diagnostic errors may occur due to rare sequence variations. It is always possible that a new, previously undiscovered (and therefore un-interrogated) CYP2C19 mutation may confer altered enzyme function in an individual, and thus lead to the rare possibility of a loss-of-function allele being erroneously called as “wild-type” (*1).
- Variants in other genes associated with drug metabolism or drug response will not be detected.

Reference Range

No reference range.

References

(1) Scott SA, Sangkuhl K, Stein CM, Hulot JS, Mega JL, Roden DM, Klein TE, Sabatine MS, Johnson JA, Shuldiner AR. Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for cytochrome P450-2C19 (CYP2C19) genotype and clopidogrel therapy: 2013 Update. *Clin Pharmacol Ther.* 2013 Sep;94 (3):317-23.

©2010 Covance CLS

207409_TMEA © 2019 American Medical Association. All rights reserved. Version 2.0