Title
Point of care CYP2C19 genotyping after percutaneous coronary intervention

Journal
The Pharmacogenomics Journal, 22(5-6)

ISSN
1470-269X

Authors
Baudhuin, Linnea M
Train, Laura J
Goodman, Shaun G
et al.

Publication Date
2022-12-01

DOI
10.1038/s41397-022-00278-4

Peer reviewed

HHS Public Access

Author manuscript

Pharmacogenomics J. Author manuscript; available in PMC 2023 June 01.

Published in final edited form as:

Pharmacogenomics J. 2022 December ; 22(5-6): 303–307. doi:10.1038/s41397-022-00278-4.

Point of Care *CYP2C19* Genotyping After Percutaneous Coronary Intervention

Linnea M. Baudhuin[1,*], **Laura J. Train**[1], **Shaun G. Goodman**[2], **Gary E. Lane**[3], **Ryan J. Lennon**[4], **Verghese Mathew**[5], **Vishakantha Murthy**[6], **Tamim M. Nazif**[7], **Derek Y.F. So**[8], **John P. Sweeney**[9], **Alan H. B. Wu**[10], **Charanjit S. Rihal**[6], **Michael E. Farkouh**[11], **Naveen L. Pereira**[6]

[1]Department of Laboratory Medicine & Pathology, Mayo Clinic, Rochester, Minnesota, USA

[2]St. Michael's Hospital, University of Toronto, Toronto, Ontario, Canada, and Canadian VIGOUR Centre, University of Alberta, Edmonton, Alberta, Canada

[3]Department of Cardiovascular Medicine, Mayo Clinic, Jacksonville, Florida, USA

[4]Department of Health Sciences Research, Mayo Clinic, Rochester, Minnesota, USA

[5]Worldwide Network for Innovation in Clinical Education and Research (WNICER) Institute, New York, New York, USA

[6]Department of Cardiovascular Medicine, Mayo Clinic, Rochester, Minnesota, USA

[7]Columbia University Medical Center, New-York Presbyterian Hospital, New York, New York, USA

[8]University of Ottawa Heart Institute, Ottawa, Ontario, Canada

[9]Department of Cardiovascular Medicine, Mayo Clinic, Phoenix, Arizona, USA

[10]Department of Laboratory Medicine, University of California, San Francisco, California, USA

[11]Peter Munk Cardiac Centre and Heart and Stroke Richard Lewar Centre, University of Toronto, Toronto, Ontario, Canada

Abstract

Loss-of-function *CYP2C19* variants are associated with increased cumulative ischemic outcomes warranting *CYP2C19* genotyping prior to clopidogrel administration. TAILOR-PCI was an international, multicenter (40 sites), prospective, randomized trial comparing rapid point of care (POC) genotype-guided vs. conventional anti-platelet therapy. The performance of buccal-

[*]Corresponding author: Baudhuin.Linnea@mayo.edu; Tel: +1 507-284-1211; Twitter: @LinneaBaudhuin (L.M. Baudhuin).

Author Contributions

LMB was the principal investigator and takes primary responsibility for the manuscript. LMB, LJT, SGF, RJL, CSR, MEF, and NLP were involved in the conception and design, collection, and compilation of data, data analysis and writing the manuscript; GEL, VM, VM, TMN, DYFS, JPS, and AHBW in data analysis, strategic suggestions, and writing the manuscript.

Competing Interests

Dr. Farkouh reports research grants from Amgen, Novartis, and Novo Nordisk. Dr. So reports unrestricted grant support from Spartan Biosciences and Fujimori Kogyo and is on advisory boards for AztraZeneca Canada, Bayer Canada, JAMP/Orimed Pharma, and HLS Therapeutics. Dr. Goodman reports research grant support and/or speaker/consulting honoraria from: AstraZeneca, Bayer, Boehringer Ingelheim, Bristol Myers Squibb, Daiichi-Sankyo/American Regent, Eli Lilly, JAMP Pharma, Pfizer, Servier, Valeo Pharma; and salary support/honoraria from the Heart and Stroke Foundation of Ontario/University of Toronto (Polo) Chair, Canadian Heart Research Centre and MD Primer, Canadian VIGOUR Centre. The remaining authors have no disclosures to report.

Clinical Trial Registration: https://www.clinicalTrials.gov (Identifier: NCT01742117)

based rapid *CYP2C19* genotyping performed by non-laboratory-trained staff in TAILOR-PCI was assessed. Pre-trial training and evaluation involved rapid genotyping of 373 oral samples, with 99.5% (371/373) concordance with Sanger sequencing. During TAILOR-PCI, 5302 patients undergoing PCI were randomized to POC rapid *CYP2C19* *2, *3, and *17 genotyping versus no genotyping. At 12 months post-PCI, TaqMan genotyping determined 99.1% (2364/2385) concordance with the POC results, with 90.7-98.8% sensitivity and 99.2-99.6% specificity. In conclusion, non-laboratory personnel can be successfully trained for on-site instrument operation and POC rapid genotyping with analytical accuracy and precision across multiple international centers, thereby supporting POC genotyping in patient-care settings, such as the cardiac catheterization laboratory.

Keywords

Percutaneous coronary intervention; pharmacogenetics; clopidogrel; CYP2C19

INTRODUCTION

Clopidogrel remains the most widely prescribed antiplatelet drug in the US and Canada (1, 2), and is administered as a prodrug, metabolized to its active form by cytochrome P450 (CYP) enzymes. Clopidogrel, administered in combination with aspirin (i.e., dual antiplatelet therapy [DAPT]), is used to reduce major adverse cardiovascular (CV) events such as myocardial infarction, stroke, stent thrombosis, and CV death in patients with an acute coronary syndrome (ACS) and following percutaneous coronary intervention (PCI). Individual response to clopidogrel is highly variable and efficacy may be dependent on a number of factors, including potential drug interactions with cytochrome (CYP) inhibitors and substrates (e.g., lipophilic statins, calcium antagonists, proton-pump inhibitors), and genetics.

One of the main enzymes that metabolizes the clopidogrel prodrug to its active form is cytochrome P450 2C19, which is encoded for by *CYP2C19* (3). While *CYP2C19* has several recognized loss-of-function (LOF) alleles and haplotypes, as annotated by the Pharmacogene Variation Consortium (Pharmvar) (4), the two most common alleles associated with clopidogrel drug action are **2* and **3* (5). These alleles are associated with lower levels of the active metabolite of clopidogrel and a marked decrease in platelet responsiveness to the drug (i.e., higher on-treatment platelet aggregation) (3, 6-8). *CYP2C19* LOF alleles have been associated with an increased rate of adverse CV events, including death, in patients taking clopidogrel (8-10).

In March 2010, the FDA issued a drug label boxed warning update to highlight the risk of clopidogrel inefficacy in CYP2C19 poor metabolizers, and suggested that tests were available to identify a patient's CYPC2C19 genotype that could be used to direct an appropriate therapeutic strategy. The TAILOR-PCI (Tailored Antiplatelet Initiation to Lessen Outcomes Due to Decreased Clopidogrel Response after Percutaneous Coronary Intervention, Clinicaltrials.gov: NCT01742117) clinical trial was initiated to determine the clinical utility of prospective *CYP2C19* genotyping of individuals prescribed clopidogrel

(11). Results from the trial demonstrated that genotype-guided therapy resulted in 34% fewer adverse events (death, stroke, myocardial infarction, and stent thrombosis) after 12 months and this effect was especially pronounced in the first 3 months with 79% fewer adverse events.

Prospective genotyping should ideally be performed in a rapid manner in order to prescribe the proper therapy in a timely fashion. Oftentimes, traditional CLIA-based laboratories are unable to return genotyping results on the same day due to clinical workflow complexities, including time involved for sample routing, DNA extraction, and sample set up and analysis. There are many logistics involved, and oftentimes it isn't feasible to perform genotyping on demand as individual samples arrive at the lab. Furthermore, many hospitals do not have molecular genetic testing on site, thereby requiring samples to be sent out to laboratory facilities that can perform molecular genetic testing. Testing in CLIA-based laboratories is typically performed by trained and certified laboratory personnel, for example clinical laboratory technologists. However, with rapid genetic testing that involves limited sample handling and closed systems, it may be possible to perform the genetic testing at the point of care (such as the cardiac catheterization patient care setting) by personnel who are not traditionally trained in laboratory testing. Herein, we describe the analytical evaluation of the Spartan® RX platform, as well as its performance characteristics during the conduction of TAILOR-PCI, providing the basis for its possible use as a POC assay by non-laboratory-trained study personnel in the cardiac catherization laboratory and other patient care settings.

METHODS

For the pre-trial analytical evaluation of the rapid genotyping assay, the Spartan® RX rapid genotyping instrument along with the companion consumable assay (Spartan® RX CYP2C19 assay, Genomadix Inc., Ottawa, ON, Canada) were placed in each of the 40 United States and international clinical trial centers. Individual buccal sample collection kits, containing individual swabs and reagent tubes were provided. The Spartan® RX system is a closed system that performs DNA extraction, amplification, and genotyping using fluorescent-labeled oligonucleotide probes and optical detection channels. System set-up and initial training of onsite testing staff was completed by a representative from Spartan Bioscience. A standardized instrument validation protocol was developed and distributed to each site.

For the volunteer testing phase, a minimum of seven healthy volunteers (maximum of ten volunteers) from each center were tested using the rapid genotyping Spartan® RX assay to detect the *CYP2C19 *2, *3,* and **17* alleles, for a total of 373 samples. Inter-assay reproducibility was tested by genotyping a subset of three volunteers who were assigned as reproducibility candidates, undergoing buccal swabs for three Spartan® RX assays at three different times. At least two instrument operators were required for all volunteer testing to test inter-operator accuracy. A Spartan® RX report for each testing incident as well as a saliva sample (Oragene saliva collection kit, DNA Genotek, Inc., Ottawa, ON, Canada) from each volunteer was submitted to determine concordance of the genotypes to a centralized Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory (Mayo Clinic, Rochester, MN, USA) for *CYP2C19* Sanger sequencing (using a clinically

validated laboratory developed test) confirmation of the genotypes obtained by the Spartan® RX platform (primer information available upon request). Prior to sequencing, saliva DNA was extracted using a Qiagen EZ1 Advanced automated DNA extraction system and a Qiagen EZ1 DNA investigator kit (Thermo Fisher Scientific Inc., Waltham, MA, USA).

A set of three control DNA samples with known *CYP2C19* genotypes (**1/*3, *2/*2,* and **2/*17*) was shipped to each of the testing sites along with a calibrated pipet and barrier pipet tips. Each site tested the DNA samples by pipetting 1 μl of the low concentration (0.01-0.1 ng/μL) DNA into the Spartan sample collection kits. A Spartan report was sent to the centralized CLIA laboratory for a concordance check. New DNA control aliquots were sent for retesting when inconclusive/failed genotype reports were received.

During the TAILOR-PCI trial, the outcomes of *CYP2C19* LOF patients receiving point of care (POC) genotype-guided (GG) anti-platelet therapy to conventional therapy with clopidogrel were compared (11). Subjects in the GG arm underwent FDA-approved POC rapid genotyping (Spartan® Rx) for *CYP2C19 *2, *3,* and **17.* A total of 5302 patients were enrolled, of which 26 patients were excluded from all analyses (19 improperly consented, five did not have PCI or failed PCI, two were duplicate randomizations). This resulted in inclusion of 5276 patients who were eligible for analysis with 2635 patients in the conventional therapy arm and 2641 patients in the genotype-guided arm by point-of-care *CYP2C19 *2, *3* and *17 analysis. After 12 months post-PCI, an alternative genotype method (TaqMan) was utilized to determine *CYP2C19* genotype in all randomized subjects, as well as to compare results with those obtained from the Spartan® Rx platform in the GG arm.

This study has been approved by the Mayo Clinical Institutional Review Board, and participants gave written informed consent.

RESULTS

In the volunteer testing phase, inclusive of all 40 sites, a total of 373 volunteer samples were analyzed for the pre-trial analytical validation of the Spartan Rx platform to detect the *CYP2C19 *2, *3,* and **17* alleles (Tables 1 and 2). Initial results demonstrated concordance in 371/373 (99.5%) samples between the POC assay and Sanger sequencing results (Fig. 1A). Root cause for discordance in the two samples was pre-analytical sample mix-up at the testing center. The two samples were re-collected and re-tested, after which concordance was observed. Inter-operator accuracy was 100%.

Sanger sequencing revealed additional heterozygous missense variants in 4 samples that were otherwise **1/*1* (c.629C>A, p.Thr210Asn and c.784G>A, p.Asp262Asn), **2/*2* (c.518C>T, p.Ala173Val), and **2/*17* (c.614T>G, p.Ile205Ser) by Spartan genotyping (Table 1). None of these variants have been described by PharmVar, and they all have been deemed to be of uncertain significance (details in Supplemental Table).

A set of 3 control DNA samples with known *CYP2C19* genotypes (**1/*3, *2/*2,* and **2/*17*) was analyzed at each of the testing sites. A Spartan report that was sent to the centralized CLIA laboratory for a concordance check demonstrated the rate of success for

genotyping the control DNA with the initial aliquots to be only 75% (30 of the 40 sites successfully genotyped the control DNA with the initial aliquots sent). Root cause of failure was determined to be the utilization of pipettors not made for pipetting the small volume (1 uL) of DNA needed for testing. New DNA control aliquots along with appropriate pipettors for 0.5-2uL volumes were sent for retesting when inconclusive/failed genotype reports were received, and 100% of the samples were successfully genotyped.

During the TAILOR-PCI study phase, there were 2641 subjects in the genotype-guided (GG) arm of the trial, with initial genotype performed by the Spartan® Rx platform (Table 2). Of these, 54 (2%) did not have a Spartan test result available and 118 (4%) had inconclusive results. In the subset of subjects (n=255) in which it was measured, the average time for the Spartan test to complete the genotyping assay was 57 minutes. For the Spartan rapid genotype, results were available within 24 hours of randomization for 99% of the 2587 subjects who underwent genotyping. Twelve months post-Spartan test, laboratory-based TaqMan genotyping was performed on each study sample. The overall concordance between the Spartan and TaqMan genotyping platforms among the 2385 subjects with both tests providing conclusive results was 99.1% (2364/2385) (Fig. 1B). Sensitivity and specificity of the Spartan analysis as compared to TaqMan ranged from 90.7 – 99.6% depending on the allele detected (Table 2).

There were 21 samples that had discordant genotypes between Spartan and TaqMan genotyping (Table 3). The discordant genotypes were not isolated to a few trial centers, but rather involved 11 of the 40 trial sites. The highest number of discordant genotypes that occurred at a single site was five samples. Out of the 21 samples, nine were called non-carrier by Spartan, but had a **2* or **3* allele by TaqMan (one of these cases was observed to be homozygous **2* by TaqMan). There were 11/21 samples that were heterozygous **2* or **3* by Spartan, but non-carrier by TaqMan. And there was one sample that was heterozygous **2* by Spartan, but homozygous **2* by TaqMan. CLIA-based Sanger sequencing was performed on 18 of the samples for which DNA was available, and it was observed that for 16/18 samples, the TaqMan genotype matched with Sanger sequencing (Table 3). There were 2 samples for which the Spartan genotype matched with Sanger (samples #7 and #8, Table 3). These samples were both **1/*1* by Taqman, but heterozygous **2* and **3* by Spartan and Sanger. The root causes of these discrepancies were not investigated further but may have been due to allelic drop-out or sample mix-up.

DISCUSSION

The TAILOR-PCI study was the first clinical trial to prospectively address the potential utility of POC GG oral P2Y12 inhibitor therapy as compared to conventional therapy with clopidogrel. In order to ensure accuracy of results and competency of testing personnel across all 40 sites, research-based validation studies prior to initiation of the trial were necessary. The analytical performance of the POC genotyping was further assessed through comparison of rapid genotyping via the Spartan instrument during the TAILOR-PCI study and follow-up genotyping via a laboratory-based TaqMan assay. The main findings of our study are, 1) greater than 99% accuracy in POC genotyping for *CYP2C19* in 2385 subjects in the genotype-guided arm of TAILOR-PCI (as demonstrated by 99.1%

concordance between Spartan and TaqMan genotyping), 2) high sensitivity and specificity of Spartan genotyping, and 3) non-laboratory trained personnel can successfully perform rapid genotyping in a POC setting. Taken together, these findings are helpful for supporting the application of rapid POC genotyping in patient care areas.

Although the FDA issued a drug label boxed warning update in March 2010 to highlight the risk of clopidogrel inefficacy in CYP2C19 poor metabolizers, and 2013 and 2022 guidelines from the Clinical Pharmacogenomics Implementation Consortium (CPIC) support genotype-guided prescribing of clopidogrel, the widespread adoption of *CYP2C19* genetic testing has not yet occurred for clopidogrel prescribing (12, 13). This may be due, in part, to the lack of recommendation for routine use of genotyping for screening purposes as per the 2011 guidelines for PCI by the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines and the Society for Cardiovascular Angiography and Interventions (ACCF/AHA/SCAI) and other clinical groups (14, 15). Other potential reasons for lack of widespread adoption of this test are the challenges of implementing rapid genetic testing in the patient care setting to provide real-time results in order to optimize treatment immediately post-PCI (10). Traditionally, *CYP2C19* genotyping has been performed in a clinical laboratory with turn-around time that could range from 1-7 days, depending on accessibility of testing. Another barrier to adoption of obtaining real-time results, at least in the United States, is due to the FDA requirement for genetic testing to be performed by a CLIA laboratory, even with FDA 510(k) clearance, such as is the case for the Spartan rapid CYP2C19 assay. However, it may be possible to place a CLIA laboratory near the point of care, for example near the cardiac catheterization laboratory. In other countries, such as Canada, the Spartan CYP2C19 system may be utilized in both patient care settings and clinical labs as long as the test is performed by a healthcare professional.

Nonetheless, the results from TAILOR-PCI and other studies have emphasized the importance of rapid, POC *CYP2C19* genotyping testing prior to P2Y12 inhibitor administration. In TAILOR-PCI, patients with *CYP2C19* LOF genetic variants in the conventional therapy arm had significantly increased cumulative ischemic events compared to those in the genotype-guided (GG) arm and the difference between the two arms in time to first ischemic event was most significant at 3 months. Another *CYP2C19* genotyping strategy-based study, POPular Genetics, demonstrated that GG P2Y12 inhibitor therapy was non-inferior to routine care with ticagrelor (16).

POC testing, or analysis of patient specimens outside of the clinical laboratory near or at the site of patient care, enables clinical decision making more rapidly than if the samples were to be sent to the clinical laboratory. Since POC testing is usually performed by clinical staff without laboratory training, staff training and competency are key to ensuring the success of POC testing especially as it relates to quality assurance of the testing (17). The RAPID GENE trial set the stage for POC genetic testing performed by non-laboratory personnel (18). It demonstrated that a strategy of rapid *CYP2C19* *2 genotyping followed by genotype-guided prasugrel administration resulted in a decreased rate of high on-treatment platelet reactivity as compared to standard therapy. The RAPID GENE trial was a successful proof of concept study, demonstrating the effectiveness of POC genotyping of a single

CYP2C19 variant in 91 subjects at a single center. Another more recent study demonstrated the utility of rapid genotyping of 781 patients at a single site (19). The TAILOR-PCI study has further expanded on the success of these trials by genotyping 3 *CYP2C19* variants in 2641 subjects at 40 international centers.

While training and competency testing of non-laboratory personnel was generally successful in the study described here, the control DNA testing phase was uniquely challenging for the on-site TAILOR PCI staff. The rate of success for genotyping the control DNA with the initial aliquots was only 75% (30 of the 40 sites successfully genotyped the control DNA with the initial aliquots sent). We hypothesize that the nurses/staff being trained to consent patients and collect buccal samples for Spartan testing were not familiar with pipetting techniques with small (1 μL) volumes of DNA. A future workaround could be to explore testing larger volumes of DNA or utilizing an alternate process for testing blinded samples. It should be emphasized that this aspect of the study did not impact the overall accuracy of the POC genotyping nor the relevance of POC testing by non-laboratory personnel.

For the TAILOR-PCI study phase, overall concordance between Spartan and subsequent TaqMan genotype was high (99.1%) among those with a conclusive POC result, demonstrating that the Spartan POC assay is reliable. For discordant samples that had follow-up Sanger sequencing (n=18), in all but two of the samples, the TaqMan genotype matched the Sanger results. The more accurate results in the TaqMan assay were not surprising since the laboratory-based TaqMan assay would be expected to be a more robust and reliable method, compared to the rapid Spartan assay. Of the three methods utilized in this study (Spartan, TaqMan, and Sanger sequencing), Sanger sequencing is the gold standard and additionally was the only method performed in a CLIA setting as a CLIA-validated laboratory developed test. Sanger sequencing of the 373 samples in the volunteer testing phase also demonstrated high concordance between Sanger and Spartan. Interestingly there were 4 samples that were called **1/*1* by Spartan but had variants of uncertain significance by sequencing. While it is unclear if these variants would actually impact clopidogrel metabolism, it does point to the issue that limited, targeted genotyping will potentially miss genetic variants that could have clinical impact. While the **2, *3,* and **17* variants in *CYP2C19* are the most common *CYP2C19* variants worldwide, there are other rare variants that affect CYP2C19 protein function, and there is considerable variation in allele frequencies amongst different ethnicities (5). The Association for Molecular Pathology and College of American Pathologists have published recommendations for CYP2C19 alleles that should be included in clinical testing, and **2, *3,* and **17* are considered tier 1 alleles, or the minimum panel of variant alleles (20). Whether or not a more comprehensive *CYP2C19* genotyping method can be implemented in a POC setting remains to be determined.

The Spartan vs. TaqMan discordance in 21 subjects was determined not to affect overall clinical outcomes of the TAILOR-PCI study. This is because approximately the same number of subject genotypes were switched from one anti-platelet to the other (i.e. 10 were Spartan wild-type and assigned clopidogrel, but should have been LOF and given ticagrelor; whereas 11 were Spartan LOF and assigned ticagrelor and should have been

given clopidogrel), and none of those 21 subjects had a primary endpoint or a bleeding event.

A recent study also demonstrated the reliability of Spartan genotyping of *CYP2C19* *2, *3, and *17 alleles in a traditional CLIA-certified lab setting (21). This study observed 100% concordance of rapid genotyping of 23 matched blood and buccal samples as well as 26 reference samples. Our study presented here expands on these findings of high analytical accuracy and precision of rapid genetic testing, but with the additional parameter of testing occurring in the patient care setting (rather than CLIA-certified lab) and at 40 international sites in a large clinical trial setting. Our study further demonstrates that on-site instrument research-based validation can be used to successfully train and ensure competency of non-laboratory-trained staff performance of rapid genotyping analysis. Additionally, the rapid genotype results from the TAILOR-PCI study were available in a timely fashion to ensure same-day genotype-guided prescribing. Taken together, this study highlights the analytical performance of a rapid POC genotyping assay and its potential for utilization in the cardiac catheterization laboratory and other patient-care settings by non-laboratory-trained staff.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding

This study was supported by the National Institutes of Health (U01HL128606 and U01HL128626 to N.L.P and M.E.F)

REFERENCES

1. Gandhi S, Zile B, Tan MK, Saranu J, Bucci C, Yan AT, et al. Increased uptake of guideline-recommended oral antiplatelet therapy: insights from the Canadian acute coronary syndrome reflective. Can J Cardiol. 2014;30(12):1725–31. [PubMed: 25475475]
2. Karve AM, Seth M, Sharma M, LaLonde T, Dixon S, Wohns D, et al. Contemporary use of Ticagrelor in interventional practice (from Blue Cross Blue Shield of Michigan Cardiovascular Consortium). Am J Cardiol. 2015;115(11):1502–6. [PubMed: 25846767]
3. Holmes MV, Perel P, Shah T, Hingorani AD, Casas JP. CYP2C19 genotype, clopidogrel metabolism, platelet function, and cardiovascular events: a systematic review and meta-analysis. JAMA. 2011;306(24):2704–14. [PubMed: 22203539]
4. Gaedigk A, Whirl-Carrillo M, Pratt VM, Miller NA, Klein TE. PharmVar and the Landscape of Pharmacogenetic Resources. Clin Pharmacol Ther. 2020;107(1):43–6. [PubMed: 31758698]
5. Botton MR, Whirl-Carrillo M, Del Tredici AL, Sangkuhl K, Cavallari LH, Agundez JAG, et al. PharmVar GeneFocus: CYP2C19. Clin Pharmacol Ther. 2021;109(2):352–66. [PubMed: 32602114]
6. Brandt JT, Close SL, Iturria SJ, Payne CD, Farid NA, Ernest CS 2nd, et al. Common polymorphisms of CYP2C19 and CYP2C9 affect the pharmacokinetic and pharmacodynamic response to clopidogrel but not prasugrel. J Thromb Haemost. 2007;5(12):2429–36. [PubMed: 17900275]
7. Hulot JS, Collet JP, Cayla G, Silvain J, Allanic F, Bellemain-Appaix A, et al. CYP2C19 but not PON1 genetic variants influence clopidogrel pharmacokinetics, pharmacodynamics, and clinical efficacy in post-myocardial infarction patients. Circ Cardiovasc Interv. 2011;4(5):422–8. [PubMed: 21972404]

8. Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, et al. Cytochrome p-450 polymorphisms and response to clopidogrel. N Engl J Med. 2009;360(4):354–62. [PubMed: 19106084]
9. Pereira NL, Rihal CS, So DYF, Rosenberg Y, Lennon RJ, Mathew V, et al. Clopidogrel pharmacogenetics. Circ Cardiovasc Interv. 2019;12(4):e007811. [PubMed: 30998396]
10. Pereira NL, Weinshilboum RM. The impact of pharmacogenomics on the management of cardiac disease. Clin Pharmacol Ther. 2011;90(4):493–5. [PubMed: 21934720]
11. Pereira NL, Farkouh ME, So D, Lennon R, Geller N, Mathew V, et al. Effect of genotype-guided oral P2Y12 inhibitor selection vs conventional clopidogrel therapy on ischemic outcomes after percutaneous coronary intervention: The TAILOR-PCI randomized clinical trial. JAMA. 2020;324(8):761–71. [PubMed: 32840598]
12. Lee CR, Luzum JA, Sangkuhl K, Gammal RS, Sabatine MS, Stein CM, et al. Clinical Pharmacogenetics Implementation Consortium Guideline for CYP2C19 Genotype and Clopidogrel Therapy: 2022 Update. Clin Pharmacol Ther. 2022.
13. Scott SA, Sangkuhl K, Stein CM, Hulot JS, Mega JL, Roden DM, et al. Clinical Pharmacogenetics Implementation Consortium guidelines for CYP2C19 genotype and clopidogrel therapy: 2013 update. Clin Pharmacol Ther. 2013;94(3):317–23. [PubMed: 23698643]
14. Levine GN, Bates ER, Blankenship JC, Bailey SR, Bittl JA, Cercek B, et al. 2011 ACCF/AHA/SCAI Guideline for Percutaneous Coronary Intervention. A report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines and the Society for Cardiovascular Angiography and Interventions. J Am Coll Cardiol. 2011;58(24):e44–122. [PubMed: 22070834]
15. Holmes DR Jr., Dehmer GJ, Kaul S, Leifer D, O'Gara PT, Stein CM. ACCF/AHA clopidogrel clinical alert: approaches to the FDA "boxed warning": a report of the American College of Cardiology Foundation Task Force on clinical expert consensus documents and the American Heart Association endorsed by the Society for Cardiovascular Angiography and Interventions and the Society of Thoracic Surgeons. J Am Coll Cardiol. 2010;56(4):321–41. [PubMed: 20633831]
16. Claassens DMF, Vos GJA, Bergmeijer TO, Hermanides RS, van 't Hof AWJ, van der Harst P, et al. A genotype-guided strategy for oral P2Y12 inhibitors in primary PCI. N Engl J Med. 2019;381(17):1621–31. [PubMed: 31479209]
17. Shaw JLV. Practical challenges related to point of care testing. Pract Lab Med. 2016;4:22–9. [PubMed: 28856189]
18. Roberts JD, Wells GA, Le May MR, Labinaz M, Glover C, Froeschl M, et al. Point-of-care genetic testing for personalisation of antiplatelet treatment (RAPID GENE): a prospective, randomised, proof-of-concept trial. Lancet. 2012;379(9827):1705–11. [PubMed: 22464343]
19. Franchi F, Rollini F, Rivas J, Rivas A, Agarwal M, Briceno M, et al. Prasugrel versus ticagrelor in patients with CYP2C19 loss-of-function genotypes: Results of a randomized pharmacodynamic study in a feasibility investigation of rapid genetic testing. JACC Basic Transl Sci. 2020;5(5):419–28. [PubMed: 32478205]
20. Pratt VM, Del Tredici AL, Hachad H, Ji Y, Kalman LV, Scott SA, et al. Recommendations for Clinical CYP2C19 Genotyping Allele Selection: A Report of the Association for Molecular Pathology. J Mol Diagn. 2018;20(3):269–76. [PubMed: 29474986]
21. Davis BH, DeFrank G, Limdi NA, Harada S. Validation of the Spartan RXCYP2C19 genotyping assay utilizing blood samples. Clin Transl Sci. 2020;13(2):260–4. [PubMed: 31664775]

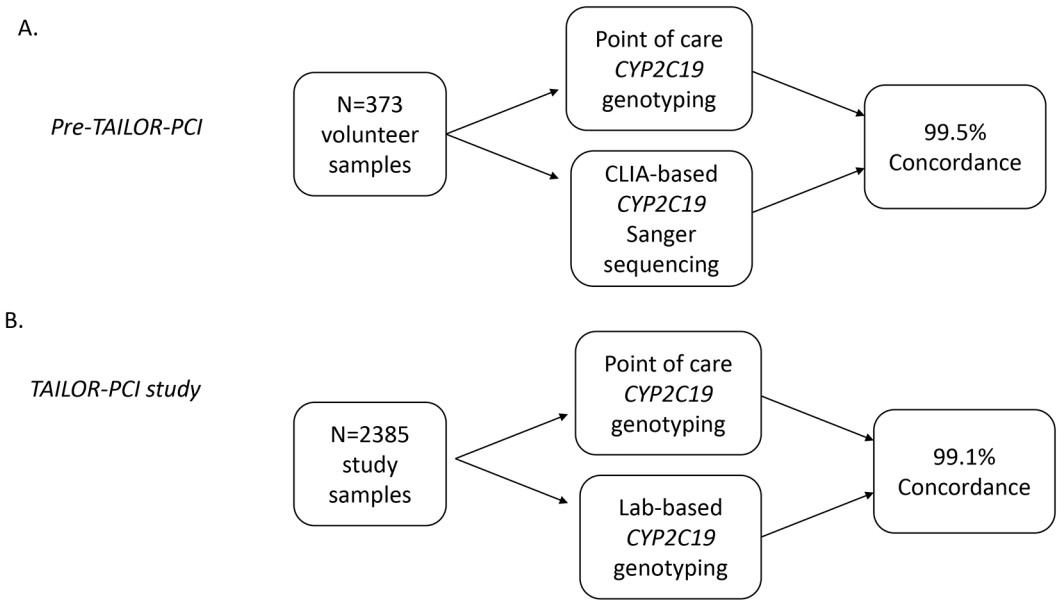

Fig. 1. Validation and Performance of Spartan Rapid *CYP2C19* genotyping in TAILOR-PCI

(A) Onsite Spartan instrument validation and training and competency of study personnel to perform rapid genotyping at 40 international sites involved in TAILOR-PCI. (B) Assessment of rapid, POC genotype results from genotype-guided study arm of TAILOR-PCI.

Table 1.

CYP2C19 Genotypes Observed in 373 Samples in Volunteer (Pre-TAILOR-PCI) Testing Phase

CYP2C19 Genotype	Number of Samples*
**1/*1* (Wildtype)	133
**1/*2*	96
**2/*2*	19
**1/*3*	7
**2/*3*	5
**3/*3*	1
**1/*17*	77
**2/*17*	23
**17/*17*	14
† Additional variants detected by Sanger sequencing	p.T210N, p.D262N, p.A173V, p.I205S

* Two samples *(*1/*1* and **1/*2*) initially showed discordant results with the Sanger sequencing not matching the Spartan RX genotype. Root cause of the discordance was shown to be due to mislabeling of the samples (sample mix-up) at the testing site.

† p.T210N, p.D262N (both with **1/*1*), p.A173V (with **2/*2*), and p.I205S (with **2/*17*) 271D

Table 2.

CYP2C19 Allele Frequency, Sensitivity and Specificity

CYP2C19 Allele	Pre-Trial	TAILOR-PCI Trial			
	Spartan n(frequency)	Spartan n(frequency)	Taqman n(frequency)	Sensitivity (%)	Specificity (%)
**2*	162 (0.22)	767 (0.16)	765 (0.16)	98.8	99.4
**3*	14 (0.02)	106 (0.02)	107 (0.02)	90.7	99.6
**17*	128 (0.17)	621 (0.13)	667 (0.14)	91.2	99.2

Table 3.

Discordant *CYP2C19* Genotypes (Spartan vs. TaqMan) and Follow-up Sanger Sequencing Results

Sample	Spartan genotype	TaqMan genotype	Sanger genotype
1	*1/*1	*1/*2	*1/*2
2	*1/*2	*1/*1	*1/*1
3	*1/*1	*1/*3	QNS *
4	*1/*1	*1/*2	*1/*2
5	*1/*2	*1/*1	*1/*1
6	*1/*1	*1/*2	*1/*2
7	*1/*2	*1/*1	*1/*2
8	*1/*3	*1/*1	*1/*3
9	*1/*2	*2/*2	*2/*2
10	*1/*2	*1/*1	*1/*1
11	*1/*2	*1/*1	*1/*1
12	*1/*1	*1/*2	*1/*2
13	*1/*2	*1/*1	*1/*1
14	*1/*2	*1/*1	*1/*1
15	*1/*2	*1/*1	*1/*1
16	*1/*1	*1/*3	*1/*3
17	*1/*1	*2/*2	*2/*2
18	*1/*2	*1/*1	*1/*1
19	*1/*2	*1/*1	*1/*1
20	*1/*1	*1/*3	QNS
21	*1/*1	*1/*2	QNS

* QNS = quantity not sufficient (insufficient sample quantity procluded further analysis)