Impact of chronic kidney disease on the pharmacodynamic and pharmacokinetic effects of ticagrelor in patients with diabetes mellitus and coronary artery disease

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Project overview

Patients with diabetes mellitus (DM) are at increased risk of atherothrombotic events. Importantly, DM is a key risk factor for the development of chronic kidney disease (CKD), which further enhances atherothrombotic risk. These observations underscore the importance of antiplatelet therapy for prevention of atherothrombotic recurrences in these high-risk patients. Clopidogrel is the most widely used platelet $P2Y_{12}$ receptor inhibitor. However, despite its clinical benefit, patients with DM and CKD frequently experience recurrent atherothrombotic events. This may be in part due to the impaired pharmacokinetic (PK) and pharmacodynamic (PD) effects of clopidogrel in patients with DM and CKD. Since both DM and CKD represent pandemic public health problems, the prevalence of which will double over the next 20 years, identifying antiplatelet agents with more favorable PK/PD profiles is of key importance.

Ticagrelor is an oral, reversible, non-competitive $P2Y_{12}$ receptor inhibitor with more potent and consistent platelet inhibition than clopidogrel. In large-scale clinical investigation, ticagrelor significantly reduced ischemic events to a greater extent than clopidogrel, a finding that was consistent also among DM patients. In patients with CKD, ticagrelor led to an even greater relative risk reduction of ischemic events, including cardiovascular mortality, compared to patients without CKD. However, to date there has been no analysis on the efficacy of ticagrelor in DM patients according to CKD status. Moreover, although PD studies showed enhanced platelet inhibition associated with ticagrelor, it is unknown how this may be affected by CKD status. Ultimately, how PK/PD profiles of different ticagrelor dosing regimens may be affected by DM and CKD status is also unknown. The proposed study is aimed to show the impact of CKD status among patients with DM and CAD on PD and PK profiles of ticagrelor used at 2 doses (90mg bid and 60mg bid) in the setting of a prospective, randomized, cross-over trial.

Background:

Diabetes mellitus (DM) and chronic kidney disease (CKD) represent pandemic diseases and a major public health concerns because of their prevalence and poor cardiovascular prognosis (1-3). DM is the most common cause of CKD and up to 40% of diabetic patients develop CKD (4). CKD was present in approximately one-third of patients with NSTE-ACS and was a powerful independent predictor of subsequent mortality (5). Importantly, patients with DM or CKD have a high thrombotic milieu and frequently experience recurrent atherothrombotic events (6-11). Multiple mechanisms may contribute to these observations, including accelerated platelet turnover rates (12,13). These observations underscore the importance of antiplatelet therapy for prevention of atherothrombotic recurrences in these high-risk patients. Clopidogrel is the most widely used platelet $P2Y_{12}$ receptor inhibitor. However, despite its clinical benefit, patients with DM and CKD frequently experience recurrent atherothrombotic events. The high atherothrombotic recurrence rates in DM and CKD patients are partly due to the inadequate response to antiplatelet agents, in particular clopidogrel, which is enhanced with the co-existence of these disorders (14-17). Therefore, identifying antiplatelet agents with more favorable pharmacokinetic (PK) and pharmacodynamics (PD) profiles is of key importance.

Ticagrelor represents an attractive strategy especially in high-risk patients, such as those with DM and/or CKD (18-20). Ticagrelor, a cyclopentyltriazolopyrimidines, is an oral, reversible, non-competitive $P2Y_{12}$ receptor inhibitor with rapid onset and offset of action and provides a stronger and more consistent platelet inhibition than clopidogrel (21-23). Although ticagrelor is a direct acting agent, ~30% of its effects are attributed to a metabolite (AR-C124910XX) derived from hepatic metabolism (23). In the large scale phase III PLATO (Platelet Inhibition and Patient Outcomes) trial conducted in ACS patients, ticagrelor, in addition to

aspirin, was associated with a significant reduction in ischemic events, driven by a reduction in myocardial infarction (MI) and cardiovascular mortality, at 1-year compared with clopidogrel (24). Such benefit was irrespective of patient management (invasive vs non-invasive). Of note, this higher efficacy was associated with no differences in overall PLATO major bleeding, although there was an increased rate of non-coronary artery bypass graft bleeding (24). A predefined subgroup analysis of patients with DM (n=4,662) showed a non-significant reduction of the rate of the primary end point (14.1% vs. 16.2%; hazard ratio [HR]: 0.88; 95% confidence interval [CI]: 0.76-1.03), which however was consistent with the overall trial results (p for interaction: 0.49) (19). The benefit of ticagrelor was enhanced in patients with worse metabolic control, as defined per HbA1c levels above the median (19). In the subgroup of patients with CKD, ticagrelor led to an enhanced benefit, with a 23% relative risk reduction of the primary ischemic end point (compared with a nonsignificant 10% reduction in patients without CKD) and an even more remarkable 4.0% absolute and 28% relative risk reduction of all-cause mortality (20).

The recently reported PEGASUS-TIMI 54 (Prevention of Cardiovascular Events in Patients With Prior Heart Attack Using Ticagrelor Compared to Placebo on a Background of Aspirin-Thrombolysis in Myocardial Infarction 54) expands upon the findings of the PLATO trial showing that prolonging dual antiplatelet therapy up to 3 years with aspirin and ticagrelor (tested at two different doses: 90 mg bid and 60 mg bid) among high-risk patients who experienced a MI in the prior 1-3 years significantly reduced the ischemic events albeit at the expense of increased bleeding complications (25). Patients required enrichment factors to be eligible for study entry which included DM and CKD; others were age >65 year, multiple prior MI, and multivessel disease which are all common features in patients with DM and/or CKD. Although both

ticagrelor doses showed a similar magnitude of clinical efficacy, the 60 mg dose appeared to have a more favorable safety profile (25). In patients with DM, the benefits of long-term use of ticagrelor was consistent with the overall trial results (HR, 0.85; 95% CI, 0.71-1.03 in DM versus HR, 0.85; 95% CI, 0.73-0.99 in non-DM; p for interaction: 0.97). There was also no apparent heterogeneity in the efficacy of ticagrelor at either dose with respect to the presence or absence of CKD (HR, 0.81; 95% CI, 0.66-0.99 in CKD versus HR, 0.88; 95% CI, 0.76-1.03 in non-CKD; p for interaction: 0.48). Indeed more insights on the efficacy of ticagrelor in patients with DM, and how this may be affected by CKD status, is being evaluated in the ongoing THEMIS (Effect of Ticagrelor on Health Outcomes in DiabEtes Mellitus Patients Intervention) Study (NCT01991795).

Significance and Rationale:

The emerging role of ticagrelor as a key agent for the secondary prevention of ischemic events underscores the need for a comprehensive understanding of its PK and PD profiles in high-risk settings such as patients with DM and CKD. These assessments will provide important mechanistic insights to recently complete and ongoing clinical outcomes studies. Importantly, these assessments will allow to better understand the aspects of ticagrelor pharmacology which may differ from other $P2Y_{12}$ inhibiting therapies. DM significantly affects the PK and PD of thienopyridine-class $P2Y_{12}$ receptor blockers (16, 26-28). However, it is unknown if DM also modulates the plasma concentrations of ticagrelor and its CYP3A4-derived metabolite (ARC124910XX). Platelets from patients with CKD are exposed to higher levels of dinucleoside polyphosphates, which can act as an agonists of the $P2Y_{12}$ receptor (29). Moreover, CKD per se

may affect disposition process, efficacy or safety of drugs (30,31). However, ticagrelor does not need biotransformation and its clearance depends minimally on renal function (23).

To date there has not been any analysis on the clinical efficacy of ticagrelor in DM patients with CKD. Moreover, although ticagrelor improved heightened platelet reactivity in DM patients, it is unknown how this may be affected by CKD status. Ultimately, whether PK/PD profiles of different ticagrelor dosing may be affected by CKD status in patients with DM is also unknown.

Study aims

The aim of the study is to assess the impact of CKD status on the PK and PD profile of ticagrelor among patients with DM and CAD. Moreover, how DM and CKD status can affect PK/PD profiles in patients treated with different dosing regimens of ticagrelor (60mg and 90 mg) will also be assessed.

Study Population

The study will be performed at the University of Florida Health Science Center at UF Health Jacksonville - Division of Cardiology. Patients will be recruited in the Cardiology Clinics of our institution and will be screened by Cardiology Research staff, who will verify that all candidates meet inclusion and exclusion criteria. Results from blood tests performed within the last 90 days will be considered valid for screening purposes. If these are not available, a blood sample will be collected for the screening phase. The research design will have common inclusion and exclusion criteria as described below.

Inclusion criteria:

- Age >18 years.
- Type 2 DM, defined according to WHO definition, on treatment with oral hypoglycemic agents and/or insulin for at least 2 months without any changes in treatment regimen;
- Angiographically documented CAD.
- On treatment with low-dose aspirin (81mg/day) and clopidogrel (75mg/day) for at least 30 days as part of standard of care.

Exclusion criteria:

- Patients with end-stage renal disease on hemodialysis.
- Use of any antiplatelet therapy (except aspirin and clopidogrel) in past 30 days.
- Use of parenteral or oral anticoagulation in past 30 day.
- Active pathological bleeding.
- History of intracranial hemorrhage with prior hemorrhage stroke.
- Blood dyscrasia or bleeding diathesis.
- Any active malignancy.
- Platelet count < $80x10^6/\mu l$.
- Hemoglobin <8 g/dl.
- Known hepatic dysfunction (known moderate and severe hepatic dysfunction).
- Hemodynamic instability.
- Known allergy or hypersensitivity to ticagrelor or any excipients.
- Pregnant / lactating females (women of childbearing age must use reliable birth control while in the study).

- Strong inhibitors of cytochrome CYP3A4 and potent inducers of cytochrome CYP3A4 (to avoid interaction with ticagrelor): ketoconazole, itraconazole, voriconazole, clarithromycin, nefazodone, ritonavir, saquinavir, nelfinavir, indinavir, atazanavir and telithromizycin.
- Patients with sick sinus syndrome (SSS) or high degree AV block without pacemaker protection.

Research design

The proposed investigation is a prospective, randomized, cross-over, open-label, PD and PK study in which CAD patients with DM on low-dose (81mg) aspirin and standard-dose (75mg) clopidogrel will be prospectively recruited. Patients will be screened during their regularly scheduled outpatient visits and informed about the study. Patients will be stratified according to CKD status into CKD and non-CKD groups. CKD will be defined according to the functional definition of the KDIGO (Kidney Disease: Improving Global Outcomes) guidelines (CKD: glomerular filtrate rate [GFR] <60ml/min/1.73m^2; non-CKD: GFR ≥ 60ml/min/1.73m^2) (32,33). The rationale for considering the functional classification to initially stratify patients is in line with clinical studies showing the increased cardiovascular risk and markedly dysfunctional platelets according to GFR strata (10,11,16). For patient stratification, GFR will be estimated through the CKD-EPI (chronic kidney disease epidemiology collaboration) equation, as this is suggested by guidelines as the most accurate method to calculate GFR, especially for values in the normal range (above 60ml/min/1.73m^2) (32,33). For comparison, GFR will also be calculated with the MDRD (modification of diet in renal disease) equation. In addition, as exploratory analysis, renal function will also be classified according to markers of kidney damage, in

particular albuminuria, in order to obtain a more comprehensive information about CKD (32,33). Albuminuria will be evaluated as albumin-to-creatinine ration (ACR) expressed in mg/g, which is approximately equivalent to albumin excretion rate. According to KDIGO guidelines, CKD will be defined as ACR >30mg/g and non-CKD as ACR ≤30mg/g (32,33). Albuminuria will be measured on random untimed spot urine samples. DM will be defined according to World Health Organization criteria and all subjects with DM will need to be on treatment with oral hypoglycemic agents and/or insulin for at least 2 months without any changes in their regimen (34).

After providing informed consent, eligible patients will be divided into CKD group and non-CKD group as described above. DM patients with CKD or without CKD will be randomized in a 1:1 fashion to one of the following treatment arms:

A. standard dose (90mg twice daily of ticagrelor) for 7-10 days (phase 1) and then cross-over to low dose (60mg twice daily of ticagrelor) for 7-10 days (phase 2)
B. low dose (60mg twice daily of ticagrelor) for 7-10 days (phase 1) and then cross-over to standard dose (90mg twice daily of ticagrelor) for 7-10 days (phase 2).

After the 2 phases of treatment (maximal treatment period 20 days) patients will resume their standard DAPT with aspirin and clopidogrel (end of study treatment).

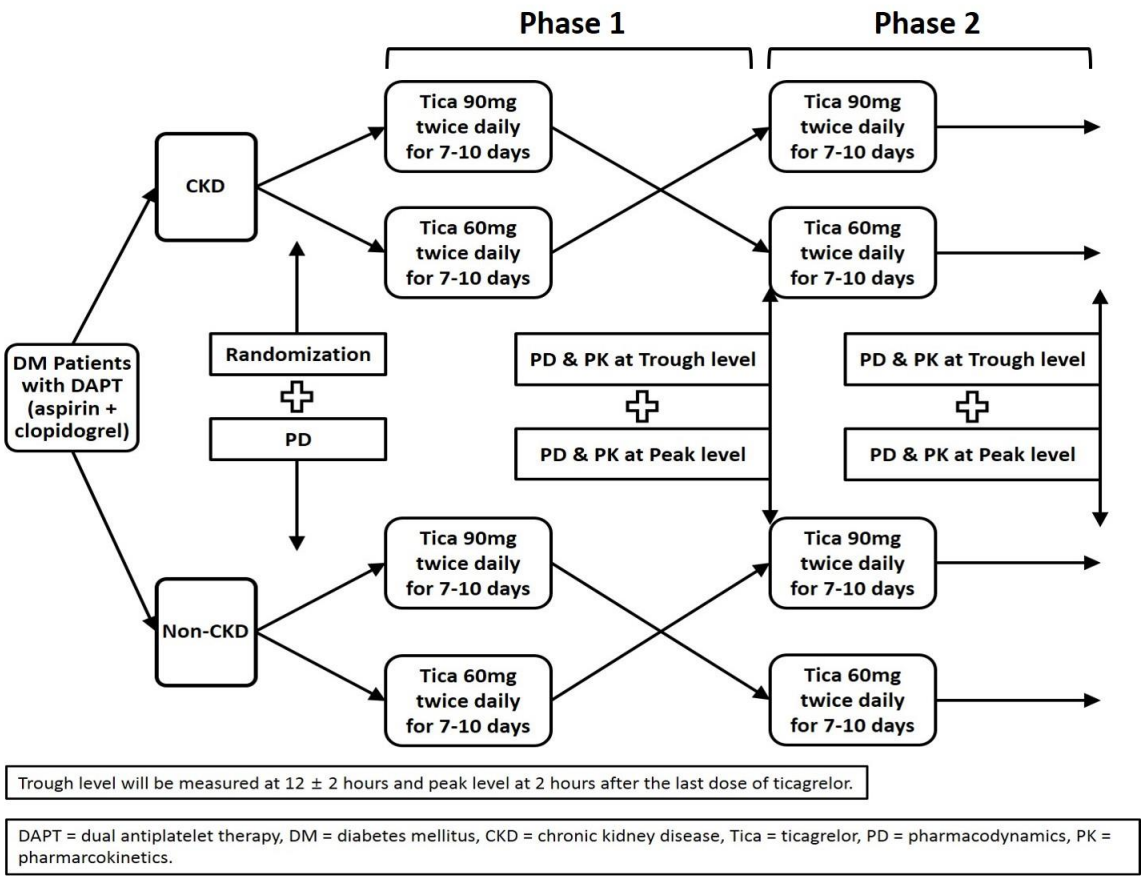
After 7-10 days of treatment, patients will come for their blood sampling in the morning hours. In particular, patients will not assume their scheduled morning dose of study medication to allow assessment of trough levels of platelet reactivity. Patients will be administered the dose of the randomized treatment while at the clinic and blood sampling will be collected after 2 hours

in order to assess peak levels of platelet reactivity. After completing the first phase of the randomized assignment, patients will initiate the second phase and start assuming assigned medication 12 hours the last dose.

Blood for sampling will be collected at the following visits (a total of 5 assessments at 3 visits):

a) baseline (24 hours after last clopidogrel MD): PD assessments
b) after 7-10 days of ticagrelor therapy (phase 1-pre-crossover): PK and PD assessment will be assessed to define trough (12 ± 2 hours after last ticagrelor dose) and peak (2 hours after ticagrelor administration) activity
c) after 7-10 days of ticagrelor therapy (phase 2-post-crossover): PK and PD assessment will be assessed to define trough (12 ± 2 hours after last ticagrelor dose) and peak (2 hours after ticagrelor administration) activity.

A flow diagram of the study is presented below.



Blood sampling: Peripheral venous blood samples (20 mL) will be drawn through a short venous catheter inserted into a forearm vein and collected in citrate and serum tubes as appropriate for assessments. The first 2-4 mL of blood will be discarded to avoid spontaneous platelet activation.

Laboratory assessments

1. Whole blood vasodilator-stimulated phosphoprotein (VASP)
2. VerifyNow P2Y12 point of care testing
3. Light-transmittence aggregometry (LTA)
4. Thromboelastography
5. Reticulated Platelets (RPs)

6. Serum thromboxane B2

7. PK assessment

Description of laboratory assays

1) *Whole blood vasodilator-stimulated phosphoprotein (VASP):* VASP phosphorylation (VASP-P) is a marker of P2Y12 receptor reactivity, which is the target for ticagrelor. VASP will be assessed according to standard protocol using labeled monoclonal antibodies by flow cytometry with the Platelet VASP-FCM kit (Biocytex Inc., Marseille, France) as previously described (16). PGE1 increases VASP-P levels by stimulation of adenylate cyclase. Binding of ADP to P2Y12 leads to Gi-coupled inhibition of adenylate cyclase. Therefore, the addition of ADP to PGE1-stimulated platelets reduces PGE1-induced VASP-P levels. If P2Y12 receptors are successfully inhibited by inhibitors, addition of ADP will not reduce the PGE1-stimulated VASP-P levels. The platelet reactivity ratio (PRI) will be calculated after measuring VASP-P levels after stimulation with PGE1 (MFI PGE1) and also PGE1 + ADP (MFI PGE1 + ADP). The P2Y12 reactivity ratio = ([MFI PGE1] – [MFI PGE1 + ADP]/[MFI PGE1]) x 100%.
2) *VerifyNow Point-of-care Testing:* The VerifyNow (VN) System is a turbidimetric based optical detection system which measures platelet induced aggregation as an increase in light transmittance (Accumetrics, San Diego, CA) and will be utilized according to manufacturer's instructions, as previously described (22). The assay is based on microbead agglutination and uses specific reagents for the pathways of interest. The VN-P2Y12 assay, by combining ADP+PGE1, measures changes in platelet function specific to P2Y12 receptor inhibitors. The assay is based upon the ability of activated platelets to bind fibrinogen. Fibrinogen-coated microparticles aggregate in proportion to the number of GP IIb/IIIa

receptors expressed. Microbead aggregation is more rapid and reproducible if platelets are activated; therefore the reagents are incorporated into the assay channel to induce platelet activation without fibrin formation. Light transmittance increases as activated platelets bind and aggregate fibrinogen-coated beads. The instrument measures this change in optical signal and reports results in P2Y12 Reaction Units (PRU).

3) *Light transmittance aggregometry (LTA)*: Platelet aggregation will be performed using LTA according to standard protocols. Blood will be collected in citrated (3.8%) tubes. LTA will be assessed using platelet rich plasma (PRP) by the turbidimetric method in a 2-channel aggregometer (chrono-Log 490 Model, Chrono-Log Corp., Havertown) as previously described (16,22). Platelet agonists will include 5 and 20 μM ADP. PRP will be obtained as a supernatant after centrifugation of citrated blood at 1000 rpm for 10 minutes. The isolated PRP will be kept at 37° C before use. Platelet poor plasma (PPP) will be obtained by a second centrifugation of the blood fraction at 2800 rpm for 10 minutes. Light transmission will be adjusted to 0% with the PRP and to 100% for the PPP for each measurement. Curves will be recorded for 6 minutes and platelet aggregation will be determined as the maximal percent change in light transmittance from baseline using PPP as a reference.

4) *Thromboelastography (CORA® system):* The CORA® system (Haemonetics Corporation, Braintree, MA, USA) is a new generation portable thrombelastography technology able to evaluate all phases of hemostasis, including time to clot formation, rate of clot formation, strength of clot and residual clot strength due to antiplatelet drugs, rate of clot lysis. Whole blood samples will be collected into non-gel heparin vacutainer tubes with ≥ 14.5 IU heparin/ mL blood, completely filled by vacuum. The PlateletMapping Cartridge are used to assess platelet function in patients who have received platelet inhibiting drugs. The

PlateletMapping assay consists of a set of agonists, ADP and AA platelet agonists together with ActivatorF, which can measure the inhibition of platelet function. This assay specifically determines the MA (Maximum Amplitude, a measure of clot strength) and the reduction in MA due to antiplatelet therapy and reports it as a percentage of reduction in clot strength. The HKH reagent, a combination of kaolin and heparinase, generates test data for the uninhibited MA resulting from thrombin activation of the blood sample, while the heparinase neutralizes the effects of heparin. The HKH test provides measures of R (Reaction time; the amount of time between the start of the test and the beginning of coagulation), K (the speed of formation of the clot from R time to a specific clot strength), Angle (the speed of clot strengthening), LY30 (Percent lysis 30 minutes after MA is finalized) and MA parameters; the activatorF test provides the contribution of fibrin to the overall strength of the clot. This test value is used in the calculation of aggregation/inhibition for MA ADP and MA AA; the AA and ADP test provide measures of MA, percent inhibition and percent aggregation.

5) *Reticulated Platelets:* Reticulated platelets (PRs) will be measured with a previously described flow cytometry assay (35,36), which is an appropriate method of measuring young platelets and platelet turnover that has been validated in multiple studies (37,38).

6) *Serum thromboxane B2*: The concentration of serum thromboxane B2 (TXB2) will be measured by using the TXB2 EIA kit (Cayman Chemical Company, Ann Arbor, MI) according to the instructions of the manufacturer (39). Briefly, samples will be diluted with EIA buffer to bring their concentrations within the range of the standard curve. No other purification will be performed on any of the samples. A standard curve will be established by serial dilution of TXB2 between 1000 pg/mL and 7.8 pg/mL using EIA buffer as the matrix.

The concentration of TXB2 in the samples will be calculated from a logistic 4-parameter fit of the standard concentrations versus percentage bound/maximum bound.

7) *Pharmacokinetic (PK) assay:* Determination of plasma concentration of ticagrelor and its active metabolite (AR-C124910XX) will be assessed from blood samples taken at the same time points of PD analysis as previously described [40]. Blood samples for measurement of drug concentrations will be drawn through the same cannula used for PD sampling. The blood will be taken into lithium heparin tubes and placed on ice until centrifugation (2800 rpm, 4°C, 10 min) within 30 min of sampling to collect the plasma, which will be transferred to plain polypropylene tubes and frozen upright at or below -20°C until analysis.

Primary and secondary objectives

The primary end point is the level of on-treatment platelet reactivity as assessed by VASP-PRI after treatment with ticagrelor 90mg bid on top of aspirin (81mg/day) for 7-10 days. The primary hypothesis of our study is that in DM patients the presence of CKD is associated with lower platelet inhibition (assessed by PRI determined by VASP-P) compared with those without CKD.

Additional objectives include:

1) The comparison of PD measures following administration of ticagrelor 90 mg between CKD and non-CKD groups at each time point assessed with PRU determined by the VerifyNow P2Y12 assay, 5 and 20 μM ADP-induced platelet aggregation by LTA and clot kinetic assessed by CORA® system.

2) The comparisons of level of platelet reactivity as assessed by multiple assays after treatment with ticagrelor 60 mg bid (on top of aspirin 81mg/day) for 7-10 days between the CKD and non-CKD groups.
3) The comparison of plasma concentrations of ticagrelor and its metabolite between the DM patients with and without CKD after 7-10 days of ticagrelor (60 and 90 mg) treatment.
4) The comparison of platelet inhibition by standard dose (90mg twice daily) versus low dose (60mg twice daily) of ticagrelor in DM patients with or without CKD.
5) The comparison of the rates of high on-treatment platelet reactivity (HPR), defined as VASP-PRI ≥50%, PRU >208, 5 μM ADP-induced maximal platelet aggregation ≥46% or ADP induced MA >47 (41,42).
6) To evaluate whether response to ticagrelor varies with the proportion of RPs in DM patients according to the CKD status.
7) To assess the effects of different levels of $P2Y_{12}$ inhibition with thromboxane levels.
8) The comparison of PD and PK measures according to the CKD classification based on markers of kidney damage (ACR).

Sample Size Computation and Power Analysis

The primary hypothesis of our study is that in DM patients the presence of CKD is associated with lower platelet inhibition compared with those without CKD after administration of ticagrelor 90 mg bid. Under the assumption of 10% absolute difference in mean PRI between CKD and non-CKD groups, with a common standard deviation of 13.0% a sample size of 37 patients per group will allow to detect a difference in platelet aggregation between the two

cohorts with a 90% power and two-sided alpha=0.05 (22). Considering the two cohorts (CKD and non-CKD), 74 patients with a valid primary end point data will be needed. Assuming a 40% rate of invalid results due to hemolysis or drop-out, up to 102 patients will need to be randomized in the study. We anticipate to consent approximately 115 patients in order to reach the target number of patients. We have chosen a 10% absolute increase of PRI by VASP-P as reference value since this has been associated with a difference in clinical outcomes (43).

Statistical analysis plan

Categorical variables will be expressed as frequencies and percentages. Continuous variables will be presented as mean ± SD or median [IQR] when appropriate. Continuous variables will be analyzed for normal distribution with the Kolmogorov-Smirnov test. Student's t-test will be used for comparison of normally distributed continuous variables. Wilcoxon test or Mann-Whitney's U test will be used for comparisons of continuous variables not following a normal distribution. An analysis of covariance (ANCOVA) method with a general linear model, using baseline variables significantly different between groups as covariates, will be used to evaluate the overall difference between groups and all between-groups comparisons, in line with other PK and PD studies (22,24). Analysis of variance (ANOVA) will be used to evaluate intragroup comparisons. In line with recommendations for cross-over study designs, treatment effects will be evaluated considering the functional parameters observed in the overall patient population after the treatment regardless of the sequence in which patients received treatment. Patients who will successfully complete at least one treatment phase of the study will be considered for comparisons. The absolute between-treatment mean differences and 95% confidence intervals will be estimated in the two groups. In order to perform an unbiased

estimation of the treatment effect, period and sequence effects, which may occur in crossover studies, will be evaluated. A p-value <0.05 will be considered statistically significant. Statistical analysis will be performed using a SPSS v22.0 software.

Publication Strategy/Additional Information

This study will be performed at the University of Florida Health Science Center at UF Health - Division of Cardiology. Patients will be recruited and consented at the Cardiology clinics in the UF Health system. Patients will be screened by cardiology research, who will verify that all candidates meet inclusion criteria. Subjects will be identified with a number and data collection sheets will all be stored in a locked area. Data will be kept for 6 years after enrollment ends to comply with HIPAA regulations. Patients will receive a handout with the names and telephone numbers of the doctors involved in the study. Patients will sign an informed consent relating to the study. Study subjects will be identified first (months 1-18): we expect to enroll 4-5 subjects monthly and complete enrollment in 18 months (total: 82 subjects enrolled). Months 19-20 will be implied for statistical analysis and months 21-22 for manuscript preparation.

We anticipate no major problems with the described protocol since the approach is a straight forward prospective study and is based on well-established methods. However, since there is limited experience with to define platelet function in this specific subset, variability may be higher than expected and we cannot currently perform a detailed sample size calculation. We anticipate adding this to the protocol after inclusion of the study population has been completed. If the sample size after one year is estimated to be too small, additional patients will be included. This approach is in agreement with recommendations for pilot investigations (44).

Possible Discomforts and Risk

In clinical trials, the most common clinical side effects of ticagrelor were dyspnea (13.8%), headache (6.5%), cough (4.9%), dizziness (4.5%), nausea (4.3%), bradycardia (4.4%) principally. Infrequent events included intracranial hemorrhage (0.3%) and severe bradycardia requiring pacemaker insertion (0.9%). Patients considered to be at high risk of bradyarrhythmic events will be excluded. The most important adverse effect associated with the use of ticagrelor is bleeding. The risk of spontaneous bleeding with ticagrelor is 2.8% (24). However, such bleeding prevalence occurred in the setting of long-term (12-15 months) trials, while our study is limited to up to 14 ± 4 days of active treatment thus reducing the risk of bleeding complications. All clinical events described above, if they were to occur, as well as death, MI, stroke, and urgent revascularization procedure with PCI or coronary artery bypass grafting will be recorded. Bleeding data will be collected using PLATO definitions (24). Clinical events will be evaluated by a local committee, comprised of 2 faculty members (2 cardiologists), not directly involved in the research. In the event of a report of a serious adverse event (major bleeding – defined as life-threatening: fatal, symptomatic intracranial hemorrhage, leading to a drop in hemoglobin of at least 5 g/dL, significant hypotension requiring intravenous inotropes, requiring surgical intervention, or requiring transfusion of 4 or more units of blood; non–life-threatening: substantially disabling, intraocular bleeding leading to vision loss, or requiring at least 2 units of blood; thrombocytopenia <50,000) the local committee will meet and antiplatelet treatment management will be managed according to physician recommendation.

Definition of Adverse Events

An adverse event is any unintended or undesirable experience that occurs during the course of the clinical investigation whether or not it is considered to be therapy related. This includes any newly occurring event or previous condition that has increased in severity or frequency since the initiation of study treatment. Adverse events will be followed until resolution while the patient remains on-study. Once the patient is removed from study, events thought to be related to the study therapy will be followed until resolution or until the patient starts a new treatment regimen.

Serious adverse events (SAE): An adverse event occurring while on study and considered related (reasonable possibility that the study treatment caused the adverse experience) to the study treatment that results in the following outcomes:

- Death

- A life-threatening adverse experience.

- A persistent or significant disability, incapacity, or is a congenital anomaly, or birth defect.

- Requires inpatient hospitalization, or prolongation of existing hospitalization.

The definition of serious adverse event also includes ‘important medical event’. Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient and/or may require medical or surgical intervention to prevent one of the other outcomes listed in the

definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

Reporting of serious adverse events

A copy of the MedWatch/AdEERs report must be faxed to AstraZeneca at the time the event is reported to the FDA. It is the responsibility of the investigator to compile all necessary information and ensure that the FDA receives a report according to the FDA reporting requirement timelines and to ensure that these reports are also submitted to AstraZeneca at the same time.

Serious adverse events that do not require expedited reporting need to be reported to AstraZeneca quarterly either as individual case reports or as line-listings. When reporting to AstraZeneca, a cover page should accompany the MedWatch/AdEERs form indicating the following:

- External Sponsored Research (ESR)
- The investigator IND number assigned by the FDA
- The investigator's name and address
- The trial name/title and AstraZeneca ESR reference number

Investigative site must also indicate, either in the SAE report or the cover page, the causality of events in relation to all study medications and if the SAE is related to disease progression, as determined by the principal investigator.

Send SAE report and accompanying cover page by way of fax to AstraZeneca's designated fax line: +1-302-886-4114.

Serious adverse events that do not require expedited reporting to the FDA need to be reported to AstraZeneca preferably using the MedDRA coding language for serious adverse events.

In the case of blinded trials, AstraZeneca will request that the Sponsor either provide a copy of the randomization code/ code break information or unblind those SAEs which require expedited reporting.

All SAEs have to be reported to AstraZeneca, whether or not considered causally related to the investigational product. All SAEs will be documented. The investigator is responsible for informing the IRB and/or the Regulatory Authority of the SAE as per local requirements.

Possible benefits

The present investigation is aimed to evaluate the differences on the functional impact of CKD on platelet inhibition by ticagrelor in DM patients. This study is not designed to evaluate differences in clinical benefit. However, differences in antiplatelet profiles will perhaps prompt further investigation of the clinical implication of this difference by means of a larger scale clinical study.

Ethical and regulatory

A copy of the protocol, proposed informed consent form, other written subject information, and any proposed advertising material must be submitted to the IRB for written approval. A copy of the written approval of the protocol and informed consent form must be

received by AstraZeneca before recruitment of subjects into the study and shipment of AstraZeneca investigational product.

The investigator must submit and, where necessary, obtain approval from the IRB for all subsequent protocol amendments and changes to the informed consent document. The investigator is to notify the IRB of deviations from the protocol or serious adverse events or unanticipated problems occurring at the site and other adverse event reports received from AstraZeneca, in accordance with local procedures.

The investigator is responsible for obtaining annual IRB approval /renewal throughout the duration of the study. Copies of the investigator's reports and the IRB continuance of approval must be sent to AstraZeneca.

Labeling and storage

The label will include the following information: Facility Name, Patient Name, Dose, Prescription Number, and Dispensing Date. In addition, the drug name will be included as "Investigational Drug". The investigational drug will be stored in the Cardiovascular Research drug room. This area has limited and restricted access.

Potential Financial Risks or Benefits

None

Conflict of Interest

Dr. Angiolillo is a consultant for Astra Zeneca, the maker of ticagrelor.

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