TITLE: Effect of Oral Minocycline in Patients with Acute Stroke – a Randomized, Open Label, Prospective Trial

Unique Protocol ID: 2024-05-01

Document Date: September 18, 2025

INTRODUCTION

Background and significance:

Stroke is the 5th leading cause of death and the leading cause of severe long-term disability in the US. The stroke-related cost is estimated to be $53 billion between 2017 and 2018 and keeps increasing every year[1]. Besides intravenous Alteplase (tPA) and Tenecteplase (TNK) has been shown to be safe, effective and recently approved by FDA for the treatment of acute ischemic stroke. Endovascular mechanical thrombectomy (EVT) has been demonstrated also as safe and effective treatment for large vessel occlusion (LVO) type of stroke in addition to IV thrombolytic therapy with tPA or TNK. In addition, numerous neuroprotective regimen trials have been conducted, including those for stroke patients. However, effective treatments or agents offering significant neuroprotective benefits in acute stroke patient remain elusive.

Minocycline is an FDA-approved second-generation, semi-synthetic broad-spectrum antibiotic that exhibits promising neuroprotective effects with multiple proposed mechanisms of action in various injury models[2]. Clinically, Lampl et al. observed that a 5-day course of oral minocycline significantly reduced neurological impairment in acute ischemic stroke in a small cohort study in 2007[3]. Hemorrhagic transformation rates did not differ by treatment groups. Acute hemorrhagic stroke accounts for about 12 – 20 % of all types of strokes, accounting for about 2 million cases worldwide annually, leading to high mortality and disability rates of up to 50%. Early initiation of oral Minocycline continues to be promising for potential neuroprotection in intracerebral hemorrhage (ICH) and possible ischemic stroke[4].

At our institution, since the Lampl study, we have used Minocycline for all eligible patients with acute strokes in clinical practice until March 2019 given its limited clinical evidence from large-scale randomized placebo-controlled trials (RCTs). Therefore, after a consensus discussion among stroke clinicians at our institution, we discontinued the program-wide routine use of Minocycline in acute stroke patient during their hospitalization in March 2019. Retrospective data analysis comparing stroke patients' outcomes measured in NIHSS (NIH stroke scale) and mortality mRS=6 (Modified Rankin Scale) between minocycline treatment period (January 2018 to March 2019) and post minocycline stoppage periods (after April 2019 and before and after COVID pandemic) demonstrated statistically significant benefits of both NIHSS and mortality (mRS =6) at discharge for patients with admission NIHSS severity between 5-20 (see below data analysis), similarly with the above reported clinical functional benefits in the Lampl study although they did not report on the mortality benefits as we had observed. In this study, we set out to conduct a prospective analysis of acute stroke patients' clinical outcomes measured by NIHSS at discharge and 90 days post stroke as primary outcome, and NIHSS at 30 days as well as mRS at discharge, 30 days and 90 days post stroke as secondary outcomes which will also include all-cause mortality (mRS=6) at discharge, 30 days and 90 days post stroke. As secondary outcome, the study will assess the rate of hemorrhagic transformation of ischemic strokes following stroke treatments such as IV thrombolytics and/or thrombectomy using ECASS II/III (European Cooperative Acute Stroke Study). The study will prospectively enroll all eligible stroke patients with moderate severity

(NIHSS 5-20 on admission) to be consecutively block randomized to either Minocycline treatment along with the best clinical treatment group or the best clinical treatment group alone (from TBD 2025 to TBD 2028), comparing the above clinical outcomes. We will have interim analysis at intervals 33%, and 66% incremental enrollment and 100% of data acquisition for safety and primary outcomes threshold examination. The results of this study may help us better understand the efficacy and safety profile of Minocycline in acute stroke treatment in both ischemic and hemorrhagic strokes, which could improve the clinical stroke care at our institution and possibly world-wide.

Primary:

- NIHSS score at discharge and on 90-day (+/- 7 days) post-stroke

Secondary:

- NIHSS at 30-day (+/- 7 days) as well as mRS at discharge, 30- (+/- 7 days) and 90-day (+/- 7 days) post stroke
- Rate of hemorrhagic transformation of ischemic strokes following stroke treatments such as IV thrombolytics and/or thrombectomy using ECASS II/III
- All-cause mortality (mRS =6) at discharge, 30- (+/- 7 days) and 90-day (+/- 7 days) post stroke

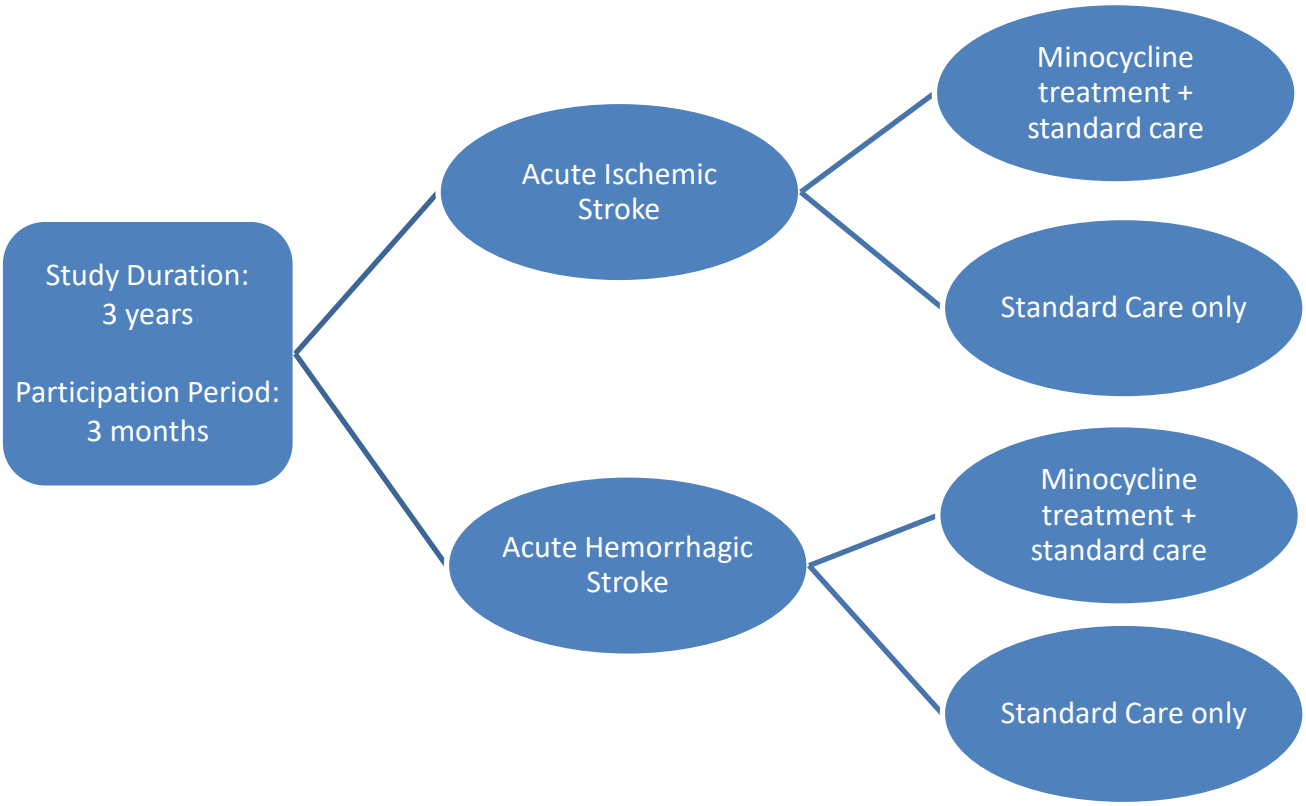
HYPOTHESIS:

Minocycline 200 mg daily oral intake for five days in addition to standard care, when administered orally in less than 24 hours after onset of acute stroke (both ischemic and hemorrhagic), improves survival and functional outcome as assessed by improvement in NIHSS score and mRS scores on discharge (including mortality, mRS=6) and on day 30, and 90 post stroke.

STUDY DESIGN:

The prospective study will enroll 1164 patients who arrive in the ER at Maimonides Hospital for suspected acute stroke. After obtaining informed consent, 1164 patients will be screened, minimum 1058 will be enrolled from TBD 2025 to TBD 2028. Consecutive block randomization will be done, and minimum of 529 patients will be assigned to either Minocycline or Standard Care arm. The minimum of 529 patients in the Minocycline arm will receive Minocycline 200 mg every 24 hours for five days in addition to standard care, while the Standard Care arm will accept only standard care. NIHSS and mRS scores will be measured on presentation, on discharge, and days 30 and 90 post stroke. A comparison will be made between both arms for their significance. Rate of hemorrhagic transformation of ischemic strokes following stroke treatments such as IV thrombolytics and/or thrombectomy will be compared using ECASS II/III (European Cooperative Acute Stroke Study) which involves follow-up brain imaging (CT or MRI) during hospitalization to detect any symptomatic intracranial hemorrhage (sICH). Statistical analysis will be done with the help of a Maimonides statistician. We will have interim analysis at intervals of subject 388 (33%), and subject 766 (66%) of enrollment/data acquisition and at 100% trial completion for safety and primary outcomes threshold examination.

SUBJECTS: Patients with clinical suspicion of stroke – both ischemic and hemorrhagic or computer tomography consistent with acute stroke consistent with WHO (World Health Organization) guidelines.



ELIGIBILITY CRITERIA:

- **Inclusion criteria**

In order to be eligible to participate in this study, an individual must meet all of the following criteria**:**

1) Age >/=18
2) NIHSS 5-20
3) Acute onset neurological deficit consistent with acute ischemic stroke or on imaging consistent with acute ischemia as defined by WHO guidelines
   OR
   Acute onset of neurological deficits with intracerebral Hemorrhage on imaging consistent with intracerebral bleed
4) The onset of neurological symptoms less than 24 hours

- **Exclusion criteria**

An individual who meets any of the following criteria is excluded from participation in this study:

1) Clinically stroke is not suspected
2) Allergic to the Tetracycline group of medications or Intolerance to Minocycline
3) Pregnancy or suspected pregnancy or breastfeeding
4) Previous history of intolerance to Minocycline
5) Acute or chronic renal failure (stage III with GFR or Creatinine threshold)
6) Any patients with contraindications to undergo CT/ MRI
7) Life expectancy less than one year or severe co-morbidities or comfort measure only (CMO) on admission
8) Pre-existing infectious disease requiring antibiotics
9) Inability to tolerate enteral medications/feeds
10) Patient/ family refusal

RANDOMIZATION: Consecutive block randomization with fixed block sizes will be used.

BLINDING TECHNIQUE: Evaluator-blinded study

DATA COLLECTION PROCEDURES:

Informed consent will be obtained from the patient or legally authorized representative (LAR) prior to any study procedures. If the patient lacks decision-making capacity, the LAR will be approached in accordance with institutional policy and state law. Consent discussions will occur in private settings with ample time for questions. The IRB-approved consent form will describe study purpose, risks, benefits, alternatives, and participant rights.

Patients presented to Maimonides Medical Center from TBD 2025 to TBD 2028 for acute stroke symptoms will be evaluated, and their eligibility for the trial will be assessed. The eligible patients

will be randomized to the Minocycline or the Standard Care arm within their respective Ischemic or Hemorrhagic group. Acute Ischemic and Hemorrhagic patients will be treated with Minocycline in addition to the standard care versus the standard care alone. Patient demographic details and information on co-morbidities will be collected. Their NIHSS and mRS scores will be measured at the time of presentation and discharge and again at 30- and 90-days post-discharge. Rate of hemorrhagic transformation of ischemic strokes following stroke treatments such as IV thrombolytics and/or thrombectomy will be assessed and recorded. All-cause mortality will also be obtained at 30 days and 90 days. The outcomes will be documented, and the results will be analyzed at intervals of subject 388 (33%) enrollment/data acquisition and subject 776 (66%) enrollment/data acquisition and 100% completion.

Data Analysis: Data analysis will be performed with the assistance of the MMC biostatistician.
All numeric variables will be summarized with mean and 95% confidence interval where appropriate and median and IQR if necessary. All categorical variables will be summarized with frequency and percentage. Continuous variables that are risk factors or demographics will be compared across groups using a t-test or Wilcoxon rank sum test. Categorical risk factors and demographics will be compared between groups using a chi-square or Fisher exact test.

The primary outcome of reduced NIHSS and mRS at discharge will be evaluated using a student's T-test. The outcome of mortality will be evaluated using a Fisher exact test. 90-day outcomes will be evaluated similarly. To control for additional risk factors, including baseline NIHSS and mRS, we will also create a generalized linear model with a normal link function for continuous outcomes, controlling for risk factors significantly different between groups. For mortality, we will use a generalized liner model with a logistic link function to control for risk factors that varied considerably between groups.

Target power	: 0.8
Actual power	: 0.800
Alpha	: 0.05
Assumed population means	: 7.1 for the placebo group and 6.1 for the Minocycline group (with an expected difference between the population's means is 1.0)
Calculated size	: 529 in each arm with a total of 1058 patients; after considering a 10% attrition rate, 582 in each arm with a total of 1164 patients.

This will include 2 interim analyses after 388 (33%) subject enrollment and data acquisition, 776 (66%), and 1164 (100%) subject enrollment and data acquisition respectively. The study will be stopped at the 1st interim analysis if the group difference in NIHSS is statistically significant at alpha = 0.0002, at the 2nd interim analysis at alpha = 0.0118. The threshold for significance at 100% data will be alpha = 0.03790 for a total study alpha = 0.05