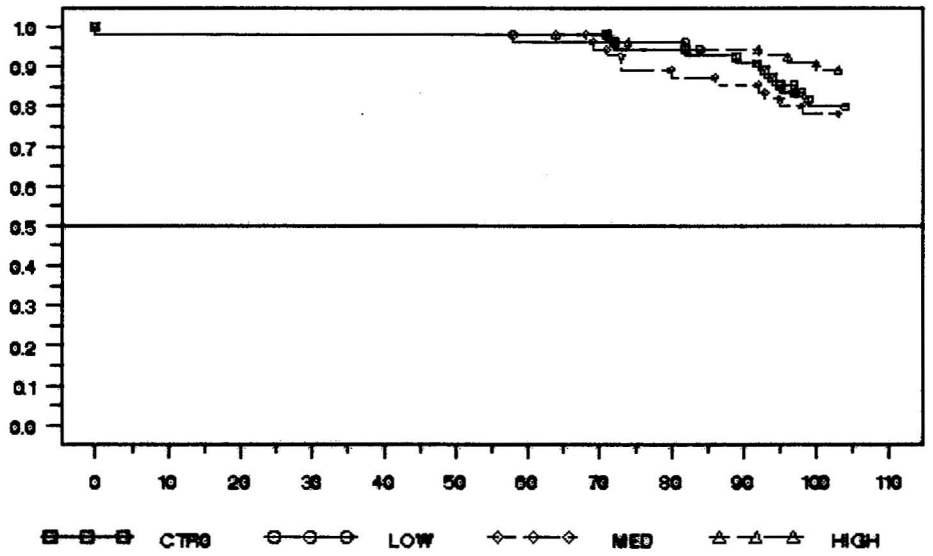
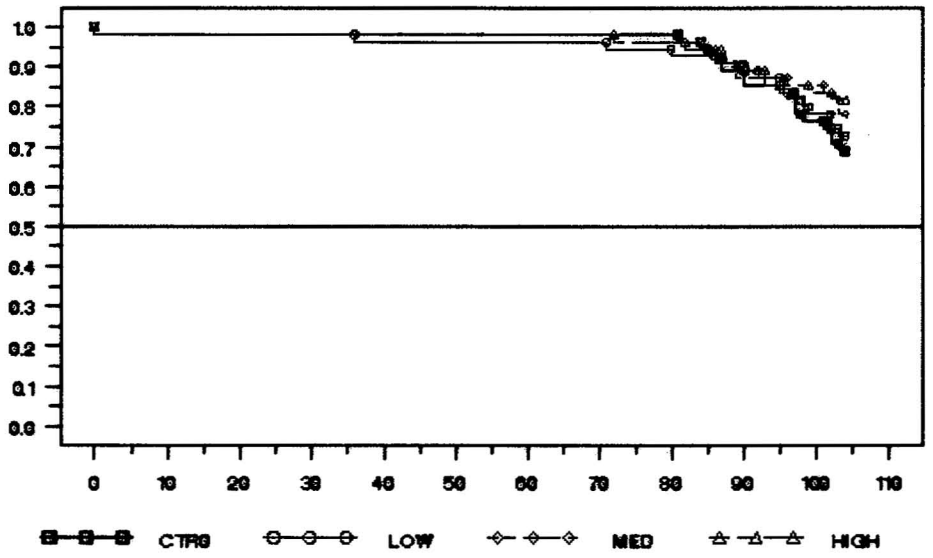
Figure 1A: Kaplan-Meier Survival Functions for Male Rats
Male Rats



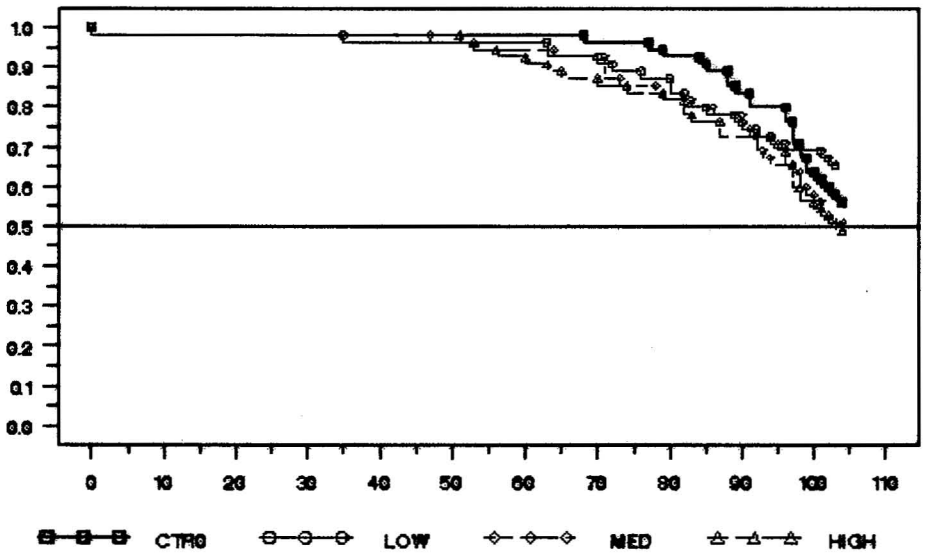
X-Axis: Weeks, Y-Axis: Survival rates

Figure 1B: Kaplan-Meier Survival Functions for Female Rats
Female Rats



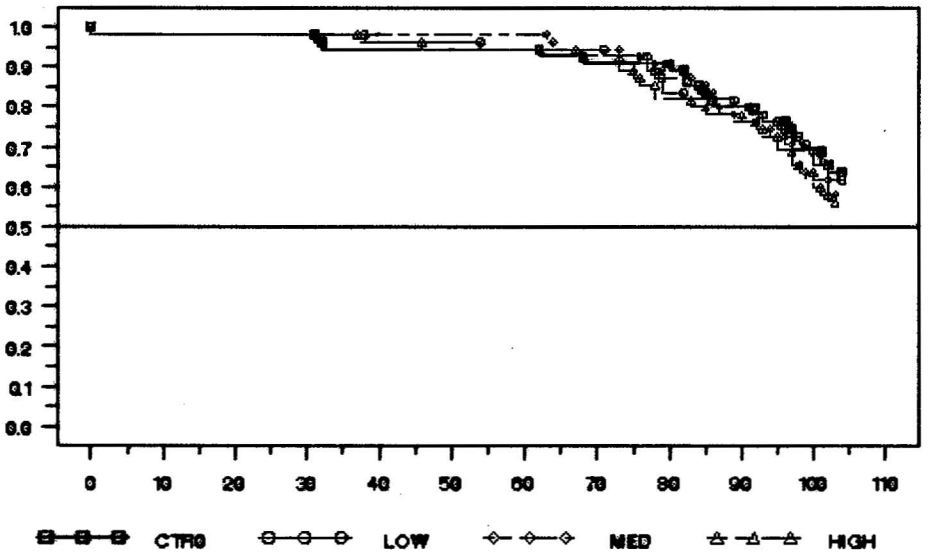
X-Axis: Weeks, Y-Axis: Survival rates

Figure 2A: Kaplan-Meier Survival Functions for Male Mice
Male Mice



X-Axis: Weeks, Y-Axis: Survival rates

Figure 2B: Kaplan-Meier Survival Functions for Female Mice
Female Mice



X-Axis: Weeks, Y-Axis: Survival rates

6. References:

1. Peto, R., M.C. Pike, N.E. Day, R.G. Gray, P.N. Lee, S. Parish, J. Peto, Richards, and J.Wahrendorf, **"Guidelines for sample sensitive significance test for carcinogenic effects in long-term animal experiments",** Long term and short term screening assays for carcinogens: A critical appraisal, International agency for research against cancer monographs, *Annex to supplement, World Health Organization, Geneva,* 311-426, 1980.

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Atiar Rahman
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Karl Lin
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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

SECONDARY STATISTICAL REVIEW AND EVALUATION

TAAL GENOMICS STUDIES

NDA/Serial Number:	22-307/S000
Drug Name:	EFFIENT (prasugrel hydrochloride) Tablets
Indication(s):	Treatment of subjects with acute coronary syndromes (ACS)
Applicant:	Eli Lilly
Medical Division:	Division of Cardio-Renal Drug Products
Statistical Review Team:	Ququan Liu, M.D., M.S., H.M. James Hung, Ph.D.
Clinical Review Team:	Karen Hicks, M.D., Norman Stockbridge, M.D., Ph.D.
Project Manager:	Meg Pease-Fye, M.S.

This secondary review provides statistical insights on the convenient genetic samples obtained in the context of a large adequate and well-controlled clinical trial - TAAL

I. Background

Study TAAL is a phase 3, multi-center, randomized, parallel-group, double-blind, double-dummy, active (clopidogrel) controlled study. Patients were randomized at the site level and stratified by clinical presentation: UA/NSTEMI versus STEMI. The study objective was to test the hypothesis that prasugrel co-administered with aspirin was superior to clopidogrel co-administered with aspirin in the treatment of subjects with acute coronary syndromes (ACS) who were to undergo percutaneous coronary intervention (PCI), as measured by a reduction in the composite endpoint of cardiovascular (CV) death, nonfatal myocardial infarction (MI), or nonfatal stroke at a mediuan of 12 months follow up – the primary efficacy endpoint. The prospectively specified primary efficacy endpoint analysis is a time to event Gehan-Wilcoxon test due to a potentially varying hazard ratio. The prospectively planned statistical test decision follows. The statistical test will be performed in the UA/NSTEMI patients at a one-sided 2.5% level. If superiority of prasugrel treatment is successfully established, the test in the all ACS patients will be performed at the same alpha level.

II. Genetically voluntarily consented (GVC) patients

In Study TAAL, the subset of patients who consented to the genetic samples in the ITT patients are summarized in Table 1. Approximately 20% to 25% of patients voluntarily consented to the genetic study. Of those, 86% to 87% are GVC evaluable (GVCE) patients. The derivation of the GVC evaluable patients (GVCE) can be found in APPENDIX. These GVCE data are approximately 17% to 21% of the ITT patients and are used by the sponsor and the statistical reviewer (Mr. Ququan Liu) for the primary efficacy analysis.

Table 1. Number (percentage) of patients in ITT who are GVC versus GVC evaluable

	UA/NSTEMI		STEMI		All ACS	
	Prasugrel	Clopidogrel	Prasugrel	Clopidogrel	Prasugrel	Clopidogrel
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
ITT	5044	5030	1769	1765	6813	6795
GVC	1023 (20)	1049 (21)	443 (25)	428 (24)	1466 (22)	1477 (22)
GVC Evaluable	880 (17)	914 (18)	374 (21)	366 (21)	1254 (18)	1280 (19)

Source: extracted from Ms. Liu's reviewer table and the sponsor table.

Of note, the assay systems used to perform genotyping, the procedures used to determine the genotypes, the genetic group classification algorithm, the functional group classification algorithm, and genotype data quality checking based on Hardy Weinberg Equilibrium are detailed in the APPENDIX.

III. Patient characteristics

The demographic and baseline characteristics of the GVCE patients with regards to age (≥ 65 yrs %, ≥ 75 yrs %), gender (Female %), race (Caucasian %), prior TIA/stroke (%), diabetes (%), TIMI-risk-score 5-14 (%), TIMI-risk-index 27.40-94.00 (%), prior MI (%), prior PCI (%), history of CHF (%), metabolic syndrome (%), and troponin > ULN at baseline (%) are tabulated side-by-side with the ITT patients, see Table 2.

As shown in Table 2, there is a consistent trend both in UA/NSTEMI patients and in all ACS patients. A preliminary comparison between the ITT patients and the GVCE patients appears to indicate that the GVCE patients might be a slightly younger patient sample with less severe medical history and baseline characteristics (percentages of patients with less diabetes, less severe TIMI-risk score, less severe TIMI-risk-index, less prior MI, less PCI, less history of CHF, less troponin > ULN at baseline) in both the prasugrel and the clopidogrel treatment patients, but, with the exception of a slightly higher percentage of patients with metabolic syndrome in clopidogrel arm (approximately 2.5% increase) than in prasugrel arm (similar or approximately 1.5% decrease).

IV. Analysis results

The results of the genetic exploratory analysis on the primary composite endpoint of CV death, nonfatal MI, or nonfatal stroke in the GVCE patients are tabulated and compared to the ITT patients, see Table 3.

The first observation from the comparisons in Table 3 is that none of the substantially statistically significant results reported by the sponsor about a superior prasugrel effect relative to clopidogrel, in UA/STEMI ITT patients and in all ACS ITT patients, in < 75 years patient subset and in diabetics patient subsets within UA/STEMI ITT and within all ACS ITT patients, were shown in the exploratory genetic analysis with the GVCE patients. Numerically, the hazard ratio estimates pointed to either a similar to an excessive hazard in the prasugrel arm as compared to the clopidogrel arm, with the exception of a numerically reduced hazard in the GVCE diabetes subset, but, the trend is extremely uncertain due to large variability in small patient samples.

V. Discussion

In this large outcome study, TAAL, comparing prasugrel (n=6813) and clopidogrel (n=6795), the genetic voluntarily consented (GVC) patients consisted of only approximately 22% of the ITT patients. And, the GVC evaluable patients are approximately 18.6% of the ITT patients. For the genetic analysis, a patient is classified into either predicted extensive metabolizer (EMfg) or predicted reduced metabolizer (RMfg) based on combination of CYP2C19 and CYP2C9 genotypes. Details of the functional bin group classification can be found in APPENDIX. From the genetic exploratory analysis, it appears that the patient size ratio of EMfg vs. RMfg is about 2:1 in the GVCE patients.

The sponsor claimed that they pre-specified primary genetic hypothesis that focused on CYP2C19 and CYP2C9 variation combined into a predicted phenotype, the binned functional group. However, from the sponsor's submission – H7T-MC-TAAL Study Report, Section 9 of this study report, "Investigational Plan includes the overall study design and plan. This reviewer did not find the prospectively specified genetic hypothesis in the statistical analysis plan.

It is worth noting that these differences are only observational, as the ITT patients were not stratified by the EMfg/RMfg. The GVCE patients are essentially a convenient sample from the ITT patient data as the genetic study objectives with respect to the primary efficacy endpoint and the key secondary efficacy endpoints of the large clinical study TAAL were not considered in the study design. Participation of the genetic study was voluntary. None of these observed numerical trends leads to any meaningful statistical analysis.

Any statistical analyses using the GVCE patient data are considered mainly exploratory. For instance, the non-replicated genetic analysis results make any further interpretation of the genetic exploratory analysis very difficult. Thus, the apparent interaction in prasugrel effect between the EMfg and RMfg subgroups (that is, prasugrel appears to show a numerically better effect than clopidogrel in the primary efficacy composite of CV death,

nonfatal MI, or nonfatal stroke in the RMfg genetic subset, but, the opposite trend seen in the EMfg genetic subset), is considered an exploratory attempt.

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Office of Biostatistics
Office of Translational Sciences
Center for Drug Evaluation and Research

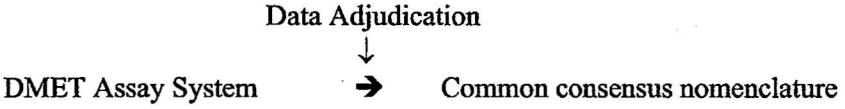
APPENDIX

Genotyping and determination of genotypes

The genes of interest for clinical outcomes evaluation are the cytochrome P450 (CYP450) enzymes 2C19 and 2C9. Determination of relevant CYP genetic variation include determination of genotypes, translation of these genetic variants into a common consensus nomenclature known as star alleles, and an adjudication process to resolve issues related to translation of variants into the common consensus nomenclature. The adjudicated data is the basis for statistical analyses investigating relationships between CYP2C19 and CYP2C9 genes and the clinical outcomes.

The primary method of generating genotypes for these genes encoding was the Drug Metabolizing Enzyme and Transporter Gene (DMET) assay system, a multiplexed genotyping platoform co-developed by Eli Lilly and Company and Affymetrix/ParAllele. Genetic variants of CYP450 2C19 and 2C9 that underwent FDA approved assay validation serves as the primary DMET genotype dataset utilized in study TAAL. Genes and alleles of primary interest measured by the DMET assay system for CYP450 2C19 are *1A, *2A, *3, *4, *5A, *6, *7, *8, *9, *10, *12, *13, and *14, and for CYP450 2C9 are *1A, *2A, *3A, *4, *5, *6, *8, *9, *10, *11A, *12. According to the sponsor, these genes/alleles are listed in order of hypothesized contribution to variability in clopidogrel metabolism. Of note, CYP2C19*17 is a genetic variant that is not available on the current DMET assay system. This assay analysis, when warranted, supplemental genotypes were generated by exon-specific polymerase chain reaction (PCR) amplification followed by standard restriction fragment length polymorphism (RFLP) analysis by gel electrophoresis or direct gene sequencing. The sponsor used validated assays performed in a GLP compliant Clinical laboratory Improvement Amendments (CLIA)-certified laboratory when such supplemental genotyping and sequencing data are needed. For both genes, *1A is the wild-type allele and the remaining alleles are considered polymorphic variants.

Data adjudication, shown in the brief schematic of genetic data flow below, is a process to resolve no call results from genotyping procedure of DMET assay system. The sponsor reported that the DMET assay system has genotyping call accuracy of > 97%.



Hardy Weinberg Equilibrium

The sponsor performed the Hardy Weinberg Equilibrium (HWE) analysis for each allele and by ethnicity. A p-value cutoff of 0.001 was used to flag the concern of the quality of genotyping assays. For CYP2C19 and CYP2C9 genes, all calculated p-values were larger than 0.001, thus, the genotype data were acceptable for further genetic exploratory analyses.

Genetic Classification

For a single gene, two approaches to classifying patients were used: a 2-group classification and a 3-group genetic classification (gc).
For the 3-group classification, the activity of each enzyme was ranked from highest to lowest into genetic classifications of predicted metabolic phenotype (Wilkinson 2005; Lynch and Price 2007):

EMgc: extensive metabolizers (normal enzyme activity)
IMgc: intermediate metabolizer
PMgc: poor metabolizer (2 alleles with little or no activity).

The 2-group classification scheme, based on the frequency of observed genotypes and the number of subjects used in the analyses, classifies a patient into either predicted normal metabolic phenotytpe (EMgc) or predicted reduced metabolizer phenotype (RMgc).

Functional Group Classification

For the purpose of classifying a patient into a binned functional group (fg), the sponsor used combined CYP2C19 and CYP2C9 predicted metabolic phenotypes. They considered either a 2- or 3- binned functional group classification. The sponsor indicated that the goal of this classification was to identify subjects who were likely, on a genetic basis, to be able to adequately metabolize clopidogrel, permitting more direct comparisons between subjects capable of activating either prasugrel or clopidogrel.

EMfg: normal or extensive metabolizers
IMfg: normal for CYP2C19 but carried reduced-function alleles for CYP2C9
RMfg: at least one reduced-function allele in CYP2C19, regardless of CYP2C9 genotype

The frequency of binned functional groups: combined CYP2C19 and CYP2C9 predicted metabolic phenotype between prasugrel and clopidogrel in the genetic subset are summarized below. The genetic subset consists of those consented to the TAAL study, and who provided informed consent and blood samples for voluntary genetic testing, abbreviated as GVC patients.

Frequency of binned functional groups in GVC patients: combined CYP2C19&CYP2C9

Functional bin group (fg)	Prasugrel N (%)	Clopidogrel N (%)
Total	1448	1459
EM	839 (58%)	876 (60%)
IM2C9	194 (13%)	179 (12%)
IM2C19	340 (23%)	317 (22%)
PM	75 (5%)	87 (6%)

Source: Sponsor Table 11.5 in the TAAL study report