SUPPLEMENTAL MATERIAL

eAppendix 1: Letter Soliciting Expert Input

Dear ___:

We have conducted an extensive literature review on an area of major controversy that surrounds the 2013 ACC/AHA Cholesterol Guidelines – whether early treatment of elevated LDL results in greater relative risk reductions than waiting until CV is already moderately elevated. We have conducted an extensive literature review, but we ask your help, as an expert in the field of cholesterol treatment, in identifying clinical evidence that we might have missed in our literature. This is particularly important given how extensive the cholesterol evidence is, and how little clinical evidence we have found addressing this issue.

We have limited our literature review to 1) randomized controlled trials, 2) high quality quasi-experiments, and 3) high-quality observational longitudinal studies (cohort studies and case-controlled studies). Below we briefly list three key arguments we have found on the early LDL treatment hypothesis, along with the supporting evidence referenced:

- ***Those at lower CV risk get more benefit per amount of LDL reduction.***
    - o Evidence found: Only evidence found: 2012 Cholesterol Treatment Trialists meta-analysis, however, we cannot find even a hint of this finding within any clinical trial, even though many should have moderate statistical power to do so if cardiac risk was evaluated as a continuous risk factor. Whenever this has been addressed within a trial, however, no evidence of this finding can be found.
    - o Problem: 1) The meta- analysis ignored strong heterogeneity between trials and failed to distinguish between within trial effects (strong evidence) vs. between trial effects (weak evidence), and 2. The approach taken in the meta-analysis was not able to deal with early termination studies appropriately (exclusion of such studies resulted in the results no longer being statistically significant).
    - o Question: Are you aware of any individual randomized controlled trials that have found that lower CV risk subjects have a greater relative treatment effect than higher risk subjects (even if not statistically significant)? Are you aware of any reports of the CTT group addressing these concerns in subsequent re-analyses?

• ***Genetic studies have suggested that long-term LDL reduction is needed to achieve full benefit:***

o Evidence found: Genetic studies have found that those with mutations that reduced LDL levels over selected controls, suggesting that a lifetime of lower LDL may result in a greater relative risk reduction than if one waited to start a statin later in life than found in statin trials.

• Jonathan Cohen, et al. Sequence Variations in PCSK9, Low LDL, and Protection against Coronary Heart Disease. NEJM 2006

• Brian Ference, et al. Effect of Long-Term Exposure to Lower Low-Density Lipoprotein Cholesterol Beginning Early in Life on the Risk of Coronary Heart Disease. JACC 2012

• Sekar Kathiresan, et al. Polymorphisms Associated with Cholesterol and Risk of Cardiovascular Events. NEJM 2008

o *Problems:* 1. Garber AM, et al (Circulation 1997) reported that in the first 2 years after statin therapy that statins' treatment effect was lower than expected, but that subsequently, statins achieved the level of risk reduction that was very close to that expected by having had that LDL all one's life (suggesting that maybe only treating 2-3 years early is all that is needed to achieve the full effect of LDL lowering), 2)presence of the genetic mutation was not an independent predictor of CV risk, over traditional risk factors, in the one study that evaluated this, 3) these results would suggest that there should be an age*LDL interaction (i.e., the longer you've had your naturally elevated LDL the higher the relative risk, which is not the case).

o *Questions:* 1. Are you aware of other evidence supporting or countering the finding reported by Garber et al (that after 2-3 years statins give as much benefit as a naturally lower LDL)?, 2. Are you aware of any clinical study finding that an LDL lowering mutation is an independent CV risk factor?, 3. We did not find any true Mendelian randomization studies (a natural true experimental design that compares those receiving the mutation with those having a parent with the mutation but not receiving the mutation). Are you aware of any such studies?

• ***Clinical trials have a "legacy effect", in that those treated more intensively during the 4-5 years of a clinical trial have a lower than expected hazard rate when they stop a statin, and for those continuing a statin, they have a lower hazards than controls who started a statin after trial cessation:***

o Evidence found: Long-term follow-up of the West of Scotland Coronary Prevention Study, and of the Heart Protection Study.

• Ford et al. Long-term follow-up of the West of Scotland Coronary Prevention Study. NEJM 2007

- Heart Protection Study Collaborative Group. Effects of 11-yearmortality and morbidity of lowering LDL cholesterol with simvastatin for about 5 years in 20,536 high-risk individuals: a randomized controlled trial. Lancet 2011

o *Problem:* This appears to be a misunderstanding of these trial results. As correctly pointed out by the HPS investigators, these studies found a statistically significant cumulative risk reduction, but this cumulative risk reduction was in steady decline since there was no legacy effect (i.e., event rates almost immediately reverted to the expected hazards, without any residual effect from earlier treatment, once statin therapy equalized between groups after the trial period ended).

o Question: Are you aware of any long-term follow-up studies that have illustrated a true legacy effect?

Thank you so much for your time and attention. Given the controversy over the 2013 AHA/ACC Guidelines, a complete and thorough review of the clinical evidence for or against the early LDL hypothesis will be a valuable service to the practicing community.

Sincerely,

Kori Sauser, MD, MScRodney Hayward, MD

Robert Wood Johnson Foundation Clinical Scholars Program University of Michigan

eAppendix 2: Proposed Analyses using CTT data

1. To assess CV risk-associated heterogeneity in treatment effect (HTE): *Consideration of cardiovascular (CV) risk as a continuous variable:* Although not a source of true bias, dividing the CV risk groups into categories reduces subgroupcomparison power substantially[1] and is not consistent with the stated hypothesis – that as CV risk increases the proportion of patients who already have extensive atherosclerosis increases. By this hypothesis, the decline in statin RRR should be monotonic. Therefore, an analysis of the CTT database limited to studies with substantial heterogeneity in CV risk and treating CV risk as a continuous factor could test for an interaction between estimated CV risk and a statin's RRR (predicted CV risk * % LDL reduction, with examination of a quadratic function to test for a gradually diminishing of the interaction effect).[2] This approach is both consistent with the underlying hypothesis and yields much greater statistical power.

2. To assess whether a given statin's RRR varies as a function of baseline LDL: *Consideration of LDL risk reduction as a multiplicative effect:* Re-analysis with standardization of studies by percent LDL reduction rather than absolute LDL reduction would better capture the multiplicative nature of the change in risk with LDL reduction. Use a multilevel model allowing for random slopes of baseline LDL to assess within versus between trial evidence of variation in RRR by baseline LDL. Alternatively, this could be examined qualitatively by examining the interaction of baseline LDL (optimally as a linear variable with a quadratic function) and the RRR of the statin used for each study individually.

3. To assess CV risk-associated heterogeneity in treatment effect (HTE): *Only include clinical trials with substantial heterogeneity in baseline risk to facilitate within-study (i.e., experimental) comparisons of heterogeneity of treatment effect (HTE) by CV risk:* HTE refers to different patient groups experiencing varying treatment effects –in this case, a better RRR in lower-risk patients.[3] HTE based on between-trial differences is an observational finding (weaker), but experimental (stronger) when HTE is based on within-trial differences.[4,5] The CTT analysis provides observational evidence because there were substantial between-trial differences in the amount of CV RRR demonstrated per LDL reduction.[1,6–24]
Many statin trials had predominantly low-moderate CV risk or predominately high CV risk study subjects. An analysis of the CTT data that is restricted to the 8 studies with good dispersion of subjects across risk groups[8–11,13,16,17,22] may better evaluate for HTE by CV risk, that is, whether the RRR per amount of LDL reduction differs between lower and higher CV risk patients. Also, when examining for HTE, studies with relative homogeneity in the examined patient attribute (in our case, CV risk) lack sufficient variance and should be excluded,[3] which was not done.

This approach is analogous to the method of propensity score matching. Propensity score matching is used in the analysis of observational data to eliminate the effect of poor balance between comparison groups. For example, if some of those receiving

treatment A in an observational study are too dissimilar than any of those receiving treatment B, or vis versa, it is essential to remove those observations to achieve balance between comparison groups. Although within group trial HTE analyses are represent true experimental analyses, between trial comparisons are observational. Therefore, analogous to propensity score matching, only using trials that had both lower and higher CV risk subjects provides balance between trials by eliminate comparisons of populations that are too different to allow a fair comparison. especially important given the large between-study HTE in CV risk reduction by amount of LDL reduction observed in statin clinical trials.

Limiting the analysis to those trials with a broad range of CV risk would provide this balance between trials. And although the number of trials examined is reduced, the power of the analysis should be strong both given the excellent heterogeneity in CV risk across the trials, and by examining CV risk a continuous variable.[3,8] This analysis should use a random effects model that accounts for clustering by trial to account for between-trial heterogeneity. And reported results should include the coefficients for treatment arm, baseline CV risk, and the interaction term between the two for each of the 8 statin trials in order to provide further transparency regarding the consistency of the HTE by CV risk effect.

eAppendix 3: Potential analyses that could provide evidence for or against the main two competing hypotheses regarding the genetic studies

As we discussed in the main paper, we concluded that the genetic studies provide strong grade B+ evidence that a lower LDL provides a greater relative risk reduction (RRR) than that found in the observational studies and statin trials. Unfortunately, the published genetic studies do not answer the question, "How long does LDL need to be reduced in order to obtain most or all of the hypothesized RRR?" In other words, if the genetic evidence is correct, does it take 5-10 years or 30 years to obtain the greater predicted RRR? We propose potential analyses of currently available data that may provide additional evidence on this important question.

1. See eAppendix 2 for a potential approach to examining heterogeneity of treatment effect (HTE) by cardiovascular risk in the CTT database.

2. The CTT database could examine LDL changes as a time-varying covariate. Many statin trials had substantial treatment contamination (cross-over and statin non-adherence) and this contamination often increased substantially over the course of the trial. An analysis that updates LDL separation between group annually (i.e., time-varying covariate) will reduce potential bias that may result in under-estimating the CV RRR per change in LDL on average and also could provide a more reliable assessment of whether a statin's RRR increases with number of years of treatment. For the functional form of the time-varying covariate, see #3 below.

3. Cohort studies with long-term follow up and annual lipid values could examine cumulative elevation of non-HDL cholesterol using a time-varying covariate (similar to the analyses examining LDL in this way recently been published by the Framingham group).[25]

4. Older cohort studies that have good CV risk factor information for young adults or adolescents could potentially link these data to national registry death files. For example, a study that measured CV risk factors, including non-HDL cholesterol, in the 1970s or 1980s could use registry data to examine long-term CV mortality to better examine how LDL earlier in life predicts long-term CV death. The currently available data suggests that this association is quite small, but this evidence is quite limited and does not adjust for regression dilution. An old cohort study with repeat measures of blood pressure and non-HDL cholesterol would be ideal in order to account for measurement error in these two risk factors, but errors in variable regression could also be used by employing external evidence on test-retest reliability in risk factor assessment.[26]

5. Examination of the genetic studies should evaluate whether the polymorphisms improve CV risk prediction. We could only find one genetic study that reported this examination (finding that it did not improve risk prediction),[27] but this could be examined in the other genetic literature. A single study may lack sufficient power because most patients do not have the genetic deficit. But lack of a substantive

improvement in risk prediction of an individual-level meta-analysis would raise question as to the validity of these studies' finding. Inclusion of these polymorphisms should improve predictiveness, given that no observed or unobserved factor other than LDL should be correlated with the genetic polymorphism (which is the key assumption in a valid instrumental variable analysis).

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