Appendix. Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about the PET/CT and MRI imaging procedures and analysis.

Supplement to: Fayad Z.A. et al. Rationale and design of dal-PLAQUE: a study assessing efficacy and safety of dalcetrapib on progression or regression of atherosclerosis using magnetic resonance imaging and 18F-fluorodeoxyglucose positron emission tomography/computed tomography.

18F-fluorodeoxyglucose positron emission tomography/computed tomography

Imaging. 18F-fluorodeoxyglucose positron emission tomography/computed tomography imaging of the carotid arteries and the ascending thoracic aorta is performed at visits 2 (screening), 5 (3 months), and 6 (6 months). Patients are instructed to avoid sweets or sugar-added drinks and alcohol during the 2 meals (approximately 12 hours) preceding 18F-FDG injection and to refrain from heavy or long-lasting exercise for 24 hours before the scan to reduce variability in muscle uptake of 18F-FDG. Glucose measurement is performed approximately 30 minutes before intravenous injection of approximately 15 mCi of 18F-FDG. If fasting blood glucose is >200 mg/dL, 18F-FDG will not be injected, and the PET scan will be rescheduled.

After a 2-hour 18F-FDG circulation time, patients are imaged in a head-first supine position. After CT imaging for localization and attenuation correction, a 2D, chest PET scan using 2 bed positions (each maintained for 10 minutes) to cover the aortic arch (upper limit) to the diaphragm (including inferior myocardial wall) is acquired. A 3D PET/CT image of the neck, with acquisition time of 15 minutes, follows aortic imaging.

Analysis

Images are sent to the core laboratory for analysis by an experienced reader. The TBR is calculated from the ratio of the standardized uptake value (SUV) of the artery compared with background venous activity.

$TBR = SUV_{Target}/SUV_{Background\ Venous\ Activity}$.

The vessel (right carotid, left carotid, or ascending thoracic aorta) with the highest TBR_{max} at baseline is considered the index vessel and will be followed throughout the study for the 18F-FDG-PET end points.

The TBR for the primary and secondary imaging end points will be determined as follows in the index vessel segment for each patient.

- Whole vessel mean of maximum TBR: the average maximum uptake of 18F-FDG (as assessed by TBR)
- Most diseased segment mean of maximum TBR: the average maximum TBR (the average maximum TBR of a group of 5 contiguous slices, centered on the slice with the hottest maximum TBR)—approximately 1.5 cm
- Whole vessel mean of mean TBR: the average mean SUV of 18F-FDG (as assessed by TBR)

The analysis will be performed using a "whole region of interest (ROI) approach" as described previously.[37,38]

In addition, the activity within the ROI that compose the index vessel will be analyzed individually. In that analysis, the distribution of 18F-FDG uptake (TBR) at 6 months will be compared with baseline, to assess whether treatment with dalcetrapib (compared with placebo) is associated with a leftward shift in activity. A separate analysis will also be done to account for the anticipated differential effect of therapy on inflamed and noninflamed vascular locations. To accomplish this, the effect of therapy on ROIs with TBR $\geq$1.6 will be analyzed separately from those with TBR <1.6.

Other exploratory analyses will include TBR changes over 3 and 6 months of non-index vessels, categorical assessments of axial sections, the average intensity of all index vessel slices with TBR $\geq$1.6, the average length of active segments of the index vessel with TBR $\geq$1.6, and frequency plot of all slices to perform signal distribution analysis (Bootstrap analysis). For the categorical assessments, the number of nonactive axial sections ($TBR_{max} <$ 1.6), the number of low active axial sections ($1.6 \leq TBR_{max} \leq 2.0$), and the number of highly active axial sections ($TBR_{max} > 2.0$) will be evaluated for shifts in distribution over time. For the signal distribution analysis, axial slice data for all subjects will be plotted (using a cumulative distribution function) to show a summary of the distribution for each treatment group.

Magnetic resonance imaging

Imaging. Magnetic resonance imaging of the carotid arteries and descending abdominal aorta is performed at baseline (visit 3) and at visits 6 (6 months), 8 (12 months), and 12 (24 months).

Patients are imaged in a head-first supine position. First, abdominal aorta images are obtained. After scout images for localization of the aorta, 2D dark-blood turbo spin echo (TSE; 16 slices), starting at the level of the diaphragm and extending downward, is obtained using proton density, T1 and T2 weightings.[39,40] After this, a cardiac retrospectively gated steady-state free procession cine image of 1 slice of the abdominal aorta is obtained for calculating the aortic compliance.

Carotid bifurcations are localized using phase contrast images. This is followed by acquisition of 2D TSE dark blood images of the common carotid arteries in proton density, T1 and T2 weightings similar to the aorta images. A time-of-flight bright magnetic resonance angiography sequence is then performed to obtain lumen contours. Finally, dynamic contrast-enhanced images are obtained on 1 slice using a 2D dark-blood TSE sequence after

administration of 0.2 mmol/kg of gadolinium-based contrast agent.[20]

Analysis. After magnetic resonance images are received by the core laboratory, they are transferred to a dedicated magnetic resonance workstation for analysis by an experienced observer. This observer also determines the presence or absence of the following plaque components: lipid-rich necrotic core, intraplaque hemorrhage, and calcification. Image components will be based on pixel intensity on multimodality images.[31,32]

The vessel wall boundaries are manually traced, as are the outer and inner walls of the common carotid and descending abdominal aorta, to derive vessel dimensions and morphologies, including lumen contour area, outer contour area, vessel wall area, and maximal and standard deviation of vessel wall thickness. To define a normalized parameter to facilitate comparisons across patients, a new metric obtained by dividing the mean wall area by the mean total vessel area will be used.[35]

Because the direct measurement of aortic compliance is difficult, it will be calculated by combining changes in lumen area over a cardiac cycle with diastolic and systolic BP information acquired from patients at the time of their examination. Specifically, the equation is (change in lumen area)/(change in measured blood pressure).

From the uptake curve of the contrast agent (gadolinium chelate), several parameters are derived that describe plaque inflammatory status. The area under the signal intensity versus time curve of the contrast agent uptake in the plaque is calculated as a measure of the uptake and retention of contrast agent in the plaque.[20]

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