Supplement 1

Determination of systolic blood pressure was done in a quiet environment with the cats un-sedated on 5 consecutive measurements using a non-invasive ultrasonographic Doppler method[23] during the screening examination and at each scheduled and unscheduled visit.

Analysis of total serum thyroxine concentration was done once within 1 month prior to enrollment.

Thoracic radiography was performed with right lateral and dorsal ventral views during the screening examination, D180, during unscheduled visits, and optionally on D10 and D60. The vertebral heart score was measured both from the right lateral and dorsal ventral views at every study site.[24,25] The presence of CHF was determined based on evidence of diffuse, multifocal, or focal unstructured increased interstitial pulmonary opacities and/or an alveolar pattern including air bronchograms suggestive of cardiogenic pulmonary edema.[42] The findings of an enhanced bronchial pattern, pulmonary venous congestion, left sided cardiomegaly, and pleural effusion were used as supportive evidence.

Comprehensive transthoracic echocardiography[26] using the available ultrasound units at the different investigator sites was performed on D-1, D0, D60, and D180, and during any unscheduled visit approximately 2 to 5 hours after dosing with the study medication (with the exception of echocardiography for diagnosis of HCM). Additional echocardiographic examinations focusing on LVOT obstruction were performed on D0 and D10. Echocardiography was done by a board-certified cardiologist and completed approximately within one month (≤ 31 days) prior to D0. Echocardiography for diagnosis was repeated on D0. Echocardiography was

preferentially performed in unsedated cats, but sedation could be used at the discretion of the investigator to facilitate the ultrasonographic study and avoid stress.

Cats were gently restraint and imaged from underneath. From a right parasternal long-axis 4-chamber view, the maximum cranial-caudal dimension of the left atrium (LAD) and the maximum dimension of the interventricular septum (IVSd) and the LV free wall (LVFWd) at end-diastole were measured. Presence and severity of systolic anterior motion of the mitral valve (SAM, yes/no) were assessed using 2D and color flow Doppler methods.[43] A right parasternal short-axis heart base view was used to determine the LA:Aorta ratio (LA:Ao)[44] and, at a slightly more apical imaging plane, again for IVDd and LVFWd measurements from 2D images. This view was also used to perform M-mode echocardiography of the LV to determine IVDd, LVFWd, LV dimension in systole (LVDs) and diastole (LVDd), and LV shortening fraction.[45] From a left apical 5-chamber view peak velocity in the LVOT was recorded using continuous wave Doppler.[43] From a left-apical 4-chamber view, LV diastolic function was evaluated as previously described[26] using isovolumic relaxation time (IVRT) and variables of transmitral flow and tissue Doppler velocities of the lateral mitral annulus. A composite of the data available led to 5 classes of LV diastolic function: Class 1 – normal LV diastolic function and classes 2 to 5 – abnormal diastolic function with Class 2 and 3 = flow pattern suggestive of 'delayed relaxation' with normal (Class 2) or increased (Class 3) filling pressures, respectively, Class 4 = 'pseudonormal' flow pattern, and Class 5 = 'restrictive' filling (Table S10). For statistical analyses, Classes 2 and 3 were later combined into one single category.

All echocardiograms were analyzed individually by the investigators on the day of examination. Two-dimensional measurements were done using the trailing edge-to-leading edge

method,[44] and all M-mode measurements were done using the leading edge-to-leading edge method.[44] The average of 3 measurements was used for statistical analysis.

A 6-lead surface electrocardiogram was acquired in all cats[27] and recorded at a minimum paper speed of 50 mm/s for at least 2 minutes. The presence of atrial and ventricular ectopy was documented.

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