WEBINAR: Targeting the Hallmarks of Aging to Reverse Age-Related Cardiac Dysfunction in Preclinical Mouse Model

Questions and answers from the November 18, 2020 webinar titled "Targeting the Hallmarks of Aging to Reverse Age-Related Cardiac Dysfunction in Preclinical Mouse Model"

This document includes questions we received and answered during the webinar, as well as those that we did not have time to address

1. **Has SS-31 been tested in clinical trials for heart failures or other diseases?**

   [Dr. Ann Chiao] Yes, there have been multiple clinical trials on SS-31.

   PROGRESS-HF is a trial of 4-week SS-31 (Elamipretide) on HFrEF and RESTORE-HF is a 4-week trial on HFpEF. The phase 2 result of PROGRESS-HF was published this year. The drug was well-tolerated but there were no improvement in outcomes (LVESV or EF). Phase 2 result of RESTORE has not been published.

   There is also a trial (Phase 2/3) for Barth syndrome, a disorder caused by abnormal cardiolipin and patients exhibit cardiomyopathy, which results were published this year. It was a 2-part trial with a total of 48 weeks of SS-31 treatment and it showed increased LV stroke volume as a secondary outcome. The positive outcome on cardiac function in the Barth syndrome trial may suggest that longer-term treatment is needed for HF trials.

   There are also a few other clinical trials, e.g. for mitochondrial myopathy and eye diseases.

2. **Will combining the 2 treatments (SS-31 and rapamycin) offer additive benefits? Similarly, if SS31 and mCAT are put together, would that enhance the cardiac beneficial effects- synergistic or additive?**

   [Dr. Ann Chiao] I don't have the data on the effects of combining SS-31 and rapamycin. My guess is that there will be additional improvement but it will not be completely additive of the effects of two treatment alone. This is due to the partially overlapping mechanisms of the two treatments (rapamycin also improves mitochondrial metabolism).
   If two treatments mediate protection via the same mechanism, it is likely there will not be additive benefits. When we combine SS-31 and mCAT, we did not observe additive benefits as

both treatments act by reducing mitochondrial oxidative stress and improving mitochondrial function.

3. **Since the changes in autophagy and mitochondrial biogenesis occur in the first 2 weeks of rapamycin treatment, will 2-week rapamycin treatment be enough to mediate the cardiac benefits?**

   [Dr. Ann Chiao] We plan to look into that but we don't have any data at this point. We think that if we give rapamycin for only 2 weeks and continue to monitor cardiac function, we will see some improvement later on from the transient changes in autophagy and mitochondrial improvement in the first 2 weeks. The extent of the benefit may be less than that of 10-week treatment as there may be additional mechanisms after 2 weeks of rapamycin treatment that contribute to the full benefit of 10-week treatment.

4. **What will happen after you stop the rapamycin treatment? Will the function stay improved or decline?**

   [Dr. Ann Chiao] A study from the Peter Rabinovitch lab published this year showed that the benefit persisted for up to 8 weeks after stopping rapamycin treatment.

5. **What happens to RNS levels in the left ventricle of old mice?**

   [Dr. Ann Chiao] RNS levels with cardiac aging is not as well documented as ROS but studies have shown increased nitration and nitrotyrosine modifications in old hearts, which would suggest increase RNS levels.

6. **Can the disturbed Redox homeostasis be reversed through exercise?**

   [Dr. Ann Chiao] Studies in rodent models showed beneficial effects of exercise training in redox homeostasis in aging hearts.

7. **Do mice develop atheroma with age?**

   [Dr. Ann Chiao] Wildtype mice of the mouse strains commonly used in lab are quite resistant to atherosclerosis and don't develop atheroma with age. Atherogenic diet feeding or genetic manipulations are used to generate mouse models of atherosclerosis.

8. **Can ventricular hypertrophy be reduced by free wheel running compared to treadmill since the latter may be stressful physiologically?**

   [Dr. Ann Chiao] We only performed treadmill running to assess exercise tolerance (at 4 and 8 weeks of SS-31 treatment) but not as a form of exercise training. Similar levels of hypertrophy were observed in mice with and without treadmill running, suggesting the hypertrophy we observed is not caused by stress from treadmill.

   Exercise training can induce physiological hypertrophy (without functional decline) so it is hard to tell how free wheel running will affect hypertrophy in old mice.

9. **Can rapamycin treatment reduce free radicals in the myocardium of aging mice?**

   [Dr. Ann Chiao] We detected reduced protein carbonyls in hearts of rapamycin treated old mice, suggesting reduced oxidative stress.

10. **Have you measured any improvements in vascular function as well as cardiac function?**

    [Dr. Ann Chiao] No, we did not measure vascular function in our studies. However, other groups have shown protective effects of SS-31 and rapamycin in cerebral vascular aging.

11. **As a general rule, do you always consider an increase in the E/A ratio from 1 towards 1.5 as a sign of diastolic function improvement? In the literature, my understanding is that anything above 1.5 is considered severe dysfunction, so could it be that they are on the way to a more severe dysfunction? Perhaps between 0.75 and 1.5 could also mean "pseudo normal function"? How can we discriminate between these two conditions? Do you measure e' (e prime) or atrium size in these mice?**

    [Dr. Ann Chiao] The diastolic function data presented were measured by tissue doppler imaging and are E'/A' ratio. Unlike E/A ratio by pulse-wave doppler, which can be pseudo normal and have restrictive filling (E/A>1.5), increase in E'/A' ratio is considered a sign of diastolic function improvement. We did not measure atrium size.

12. **Did you try pulsing Rapamycin on a 2-week schedule to see if the autophagy and biogenesis would continue at the 10 week or greater time points?**

    [Dr. Ann Chiao] We have not. But it will be interesting to see how autophagy, biogenesis and cardiac function change after pulsing 2-week rapamycin treatment.