Published in final edited form as:

Circulation. 2016 February 9; 133(6): e389–e390. doi:10.1161/CIRCULATIONAHA.115.019228.

Response to Letter Regarding Article, "Circulating microRNA-30d is associated with response to cardiac resynchronization therapy in heart failure and regulates cardiomyocyte apoptosis: a translational pilot study"

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We thank Sardu and colleagues for their comments. Our report demonstrates that baseline levels of miR-30d are correlated with response to cardiac resynchronization therapy (CRT), and that miR-30d was dynamically regulated by mechanical stress[1]. Moreover, miR-30d appeared to be an adaptive response, and was cardioprotective against tumor necrosis factor (TNF-α)-mediated apoptosis. Similar to our study, Marfella et al. noted differential expression of several plasma miRNAs in CRT responders versus non-responders 1 year after CRT[2]. The lack of significant overlap between the sets of extracellular miRNAs reflects some of the ongoing issues in extracellular RNA research: i) differences in patient populations and small sample sizes; ii) variances in methodology: several groups, including ours, have noted that differences in the manner of acquisition/storage of archived biofluid specimens, RNA isolation methods and platform for measurement of ex-RNAs can have

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Conflict of Interest Disclosures: None.

significant effects[3]; and iii) lack of adequate normalization strategies for ex-RNAs leading to the use of spike-ins for normalization (does not normalize for sample quality).

Nonetheless, there are several intriguing themes that emerge by comparing the two studies. Most notably, the candidate miRNAs appear to play a functional role in cellular processes relevant to cardiac remodeling. Secondly, while miR-30d was down-regulated in our study following CRT in responders, levels of miR-30d were higher in responders compared to non-responders (much like the candidates in the work by Marfella and colleagues). We focused on miR-30d in our paper, given that it was the leading candidate from our clinical cohort. Nevertheless, we agree with Sardu et al. that other members of the miR-30 family—and other extracellular miRNAs that are differentially present in responders versus non-responders—may indeed play complementary roles in cardiac remodeling. Specifically, the miR-30 family is particularly interesting, as it is altered in several models of cardiovascular diseases[4], and appears to modulate central molecular pathways in cardiac remodeling, including inflammation, apoptosis, autophagy, and the cellular response to adverse neurohormonal signaling (e.g. angiotensin II)[4, 5]. In our study, we demonstrated not only the anti-apoptotic role for miR-30d, but also showed that it blocked TNF-α-induced markers of pathological hypertrophy. Ultimately, the proof of the adaptive role for miR-30 family members in cardiac diseases will have to come from *in vivo* gain-of-function/loss-of-function experiments. Such experiments would ultimately strengthen the notion that ex-RNA biomarkers may play a functional role in disease pathogenesis—a new frontier of molecular biomarkers of disease and personalized medicine.

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