Can chronic cardiac allograft rejection (CCAR) be detected before irreversible vascular changes occur?
Noninvasive assessment of CCAR by cellular MR imaging in a rat model

Qing Ye[1], Yijen L Wu[1], Lesley M Foley[1], Parker H Mills[1], T. Kevin Hitchens[1], Fang-Cheng Yeh[1,2], Haosen Zhang[1], Danielle F Eytan[1], Li Liu[1], and Chien Ho[1]

[1]NMR Center for Biomedical Research and Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA, United States, [2]Department of Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA, United States

TARGET AUDIENCE: We believe that this study would be of interest to both clinicians and biomedical scientists interested in translating MRI techniques for clinical surveillance as well as cell tracking research.

INTRODUCTION: Recent advances in molecular/cellular MR imaging have profoundly impacted preclinical research and guided management of clinical cardiovascular disease including chronic cardiac allograft rejection (CCAR), which remains an obstacle to long-term allograft survival. The current clinical practice for diagnosis relies on invasive procedures, such as coronary angiography or intravascular ultrasound (1). These methods are less than ideal because they only detect vascular changes, such as artery narrowing or wall thickening, which at those stages are hardly reversible and too late for therapeutic intervention. The development of CCAR is a complex process that is not well understood, but a growing body of evidence supports the involvement of an inflammatory milieu contributing to the development of CCAR. Characterization of the development of CCAR could improve understanding of the process and aid in early identification of the disease. In this study, we sought to visualize CCAR lesions prior to irreversible vascular changes in a rat model by noninvasive cellular MR imaging (CMRI) with whole-heart information.

METHODS: A heterotopic abdominal working heart transplantation model in rats was used for this study where the natural configuration of pulmonary and coronary circulation is preserved in this mode (2). For CMRI, micrometer-sized iron oxide (MPIO) particles containing dragon green fluorescence were used to label immune cells (mainly monocytes/macrophages) *in vivo* via i.v. injection one day prior to initial CMRI. The accumulation of MPIO-labeled cells during CCAR lesion formation was tracked with the EKG and respiratory gated CMRI on a Bruker AVANCE 4.7 T system every 2 weeks over the time course of 4 months, and confirmed with *ex vivo* high-resolution 3D MR microscopy (MRM) on a Bruker 11.7-T/89-mm system, as well as fluorescence microscopy and pathology.

RESULTS AND DISCUSSION: Graft status was assessed via longitudinal monitoring of MPIO-labeled cell accumulation associated with CCAR lesion formation by CMRI (Fig. 1, A-C). The infiltrates appear as dark spots of hypointensity (arrows), which can be clearly seen with MRM (Fig.1 D-F). These dark spots are caused by cells labeled with MPIO particles which were confirmed by fluorescence microscopy (Fig.1 G). CMRI indicated that there are fewer dark spots observed in the allograft with mild CCAR (Fig. 2 A) compared with that of the allograft with severe disease (Fig. 2 D). Fluorescence microscopy (Fig. 2 B and E) and hematoxylin and eosin (H&E) stain (Fig. 2 C and F) on the corresponding tissue sections confirmed the CMRI results. CMRI is able to identify the lesion formation prior to irreversible vascular changes occur**.**
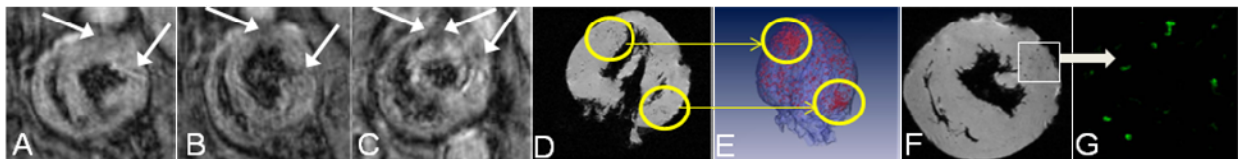

Figure1. T_2*-weighted CMRI at 4.7 Tesla show accumulation of MPIO-labeled cells associated with CCAR progression on post-operative days 14, 47, and 96 (A-C, respectively). The MPIO-labeled cells, appear as spots of hypointensity (arrows), and was confirmed high-resolution 3D MRM at 11.7T (D-F). 3D rendering provides the whole-heart perspective that lesion formation is heterogeneous (E). Co-registered MRM (F) and fluorescence microscopy (G, dragon green fluorescence) confirm that these dark spots detected by MRI are actually MPIO-labeled cells.
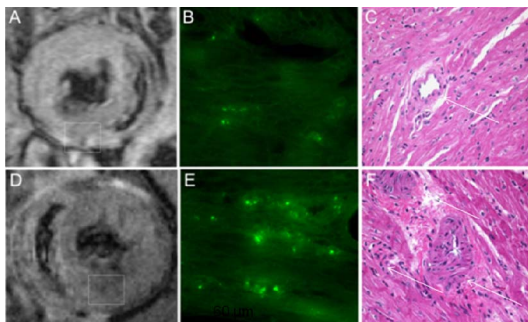

Figure 2. CMRI (left column) is able to discriminate a case of mild CCAR (A-C) prior to irreversible vascular changes from severe CCAR (D-F) and confirmed with fluorescence microscopy (middle column) and H&E stain (right column).

CONCLUSION: Our preliminary results reveal that CCAR appears heterogeneous in its early stages and the accumulation of MPIO-labeled cells can be an indicator of the lesion formation prior to irreversible vascular changes. CMRI is able to evaluate graft status non-invasively over time, which may be a reliable alternative for assessment of CCAR and potentially translatable to clinical work.

REFERENTS: (1). Mehra MR, Crespo-Leiro MG, Dipchand A, et al. *J Heart Lung Transplant* 2010;29:717-27; (2). Ye Q, Wu YL, Foley LM, et al. *Circulation.* 2008;118(2):149-56