Background: Doxorubicin and doxorubicin-trastuzumab combination chemotherapy have been associated with cardiotoxicity that eventually leads to heart failure and may limit dose-effective cancer treatment. Current diagnostic strategies rely on decreased ejection fraction (EF) to diagnose cardiotoxicity.

Purpose: The aim of this study is to explore the potential of cardiac MR (CMR) imaging to identify imaging biomarkers in a mouse model of chemotherapy-induced cardiotoxicity.

Methods: A cumulative dose of 25 mg/kg doxorubicin was administered over three weeks using subcutaneous pellets (n = 9, Dox). Another group (n = 9) received same dose of Dox and a total of 10 mg/kg trastuzumab (DT). Mice were imaged at baseline, 5/6 weeks and 10 weeks post-treatment on a 7T MRI system. The protocol included short-axis cine MRI covering the left ventricle (LV) and mid-ventricular short-axis tissue phase mapping (TPM), pre- and post-contrast T1 mapping, T2 mapping and Displacement Encoding with Stimulated Echoes (DENSE) strain encoded MRI. EF, peak myocardial velocities, native T1, T2, extracellular volume (ECV), and myocardial strain were quantified.

Results: Global peak systolic longitudinal velocity was reduced at 5/6 weeks in Dox (0.6 ± 0.3 vs 0.9 ± 0.3, p = 0.02). In the Dox group, native T1 was reduced at 5/6 weeks (1.3 ± 0.2 ms vs 1.6 ± 0.2 ms, p = 0.02), and relatively normalized at week 10 (1.4 ± 0.1 ms vs 1.6 ± 0.2 ms, p > 0.99). There was no change in EF and other MRI parameters and histopathologic results demonstrated minimal apoptosis in all mice (≈1–2 apoptotic cell/high power field), suggesting early-stage cardiotoxicity.

Conclusions: In a mouse model of chemotherapy-induced cardiotoxicity using doxorubicin and trastuzumab, advanced CMR shows promise in identifying treatment-related decrease in myocardial velocity and native T1 prior to the onset of cardiomyocyte apoptosis and reduction of EF.

Abbreviations: BLOSM, Block LOw-rank Sparsity with Motion-guidance; Ecc, Circumferential Strain; CMR, Cardiac Magnetic Resonance; DENSE, Displacement Encoding with Stimulated Echoes; Dox, Doxorubicin; DT, Doxorubicin-Trastuzumab; EF, Ejection Fraction; ECV, Extra-Cellular Volume; LV, Left Ventricle; TPM, Tissue Phase Mapping.
1 | BACKGROUND

Anthracycline-induced cardiotoxicity is an important limiting factor preventing dose-effective cancer treatment and the associated cardiac sequelae appears to be mediated through cardiac myocyte apoptosis. Anthracyclines are used in multiple hematologic malignancies and solid tumors in both adults and children. The incidence of anthracycline-induced cardiotoxicity has been estimated at 6–18% in patients treated with these agents. Combination therapies of doxorubicin and trastuzumab have been shown to exacerbate cardiotoxicity beyond what is expected with doxorubicin alone.

Current diagnostic strategies in patients at risk of cardiotoxicity primarily rely on reduced ejection fraction to make the diagnosis, which may imply a relatively advanced degree of myocardial damage. Both pre-clinical and clinical studies have suggested there is potential for cardiovascular magnetic resonance (CMR) multiparametric analysis to detect acute doxorubicin-induced cardiotoxicity, specifically through T1 and T2 myocardial tissue characterization and extracellular volume fraction (ECV) calculation. Studies using echocardiography and CMR have also suggested that global and regional circumferential and global longitudinal strain are reduced following treatment with doxorubicin.

There have been several clinical papers investigating the effects and outcomes of cardiotoxicity. However, few studies have looked at cardiotoxicity in animal models using MRI. In order to study the mechanisms underlying cardiotoxicity, animal models and cardiotoxicity studies in genetically modified mouse models are necessary. A prior study looked at effects on myocardial edema, myocardial fibrosis and LV ejection fraction due to chemotherapy in a mouse model of cardiotoxicity. They found that native T1 and T2 increased 5 weeks after chemotherapy and ECV increased along with a drop in LVEF 10 weeks after treatment. However, the authors did not look at effects of cardiotoxicity on myocardial strain or myocardial tissue velocity which the present study addresses. Myocardial tissue velocity has been studied in some patient cohorts and in a few mouse models however it has not been studied in a mouse model of chemotherapy induced cardiotoxicity.

In the current study, we have developed a multiparametric CMR cardiotoxicity examination including Displacement Encoding with Stimulated Echoes (DENSE) strain encoded MRI and tissue phase mapping (TPM) for myocardial function assessment. TPM directly measures myocardial velocities as opposed to myocardial strain, which is derived from myocardial deformation, and this technique has shown high reproducibility and interobserver agreement. We have applied this protocol in a mouse model of doxorubicin cardiotoxicity created using slow release doxorubicin pellets and hypothesize that multiparametric CMR including myocardial functional characterization will allow for the identification of doxorubicin-induced cardiotoxicity prior to cardiac myocyte apoptosis and that the advanced MR methods will be more sensitive than LVEF assessment alone. We also applied this protocol in a mouse model of combination therapy (doxorubicin and trastuzumab) to test the hypothesis that the advanced CMR protocol can detect differences between doxorubicin only vs. a combination therapy.

2 | METHODS

All animal experiments are included as part of an Institutional Animal Care and Use Committee (IACUC) approved protocol.

2.1 | Experimental design

In this study, n = 18 15–16 week old C57Bl/6 mice were included. Older mice were utilized to try and mitigate any aging effects over the course of the study period.

The animals were divided into two groups. Group 1 (Dox) consisted of n = 9 mice treated with subcutaneous doxorubicin pellets over the course of 3 weeks (week 1: 10 mg/kg, week 2: 10 mg/kg, week 3: 5 mg/kg). Thus, for group 1, we implanted two 5 mg/kg pellets in weeks 1 and 2 and one 5 mg/kg pellet in week 3. Group 2 (DT) consisted of n = 9 mice treated with 25 mg/kg dose of doxorubicin following the same schedule as the Dox group, but this group was also concurrently treated with a total of 10 mg/kg trastuzumab (3.3 mg/kg in weeks 1–3). Trastuzumab was administered via intraperitoneal injection (Weeks 1–3: 3.3 mg/kg). For both groups, the mice reached the full dose by week 5/6. Body weight was monitored weekly for all mice.

All animals were imaged at baseline and at 5/6 weeks following treatment initiation. N = 3 mice were sacrificed from the DT group and N = 4 mice were sacrificed from the Dox group at 5/6 weeks after chemotheraphy. And the remaining subgroup of mice (Group 1 n = 5, Group 2 n = 6) were imaged at 10 weeks following treatment initiation (Figure 1). Since imaging was performed at baseline before the start of treatment (control timepoint), no control/sham group was included. More details about the mouse model are provided in the Supplementary Information.
2.2 | Cardiovascular MRI

All animal CMR was performed on a 7 T Clinscan system (Bruker, Ettlingen, Germany) using a 4 channel phased array surface coil built for mouse cardiac applications. During MRI, anesthesia was maintained using 1.25% isoflurane in O₂. The MRI protocol is presented in Table 1 (scan time: 2 hours per mouse). Details about the various MRI sequences are given below and sequence parameters and analysis techniques are described in Supplementary Information:

2.2.1 | Cine MRI

Prospectively ECG and respiratory gated multi-slice steady-state free precession images were acquired covering the entire LV from base to apex (scan time = 20 min). Imaging parameters and details about analysis are given under Supplementary Information. Using the cine images, the end-diastolic volume (EDV), end-systolic volume (ESV) and ejection fraction (EF) were calculated.

2.2.2 | Tissue Phase Mapping

Tissue phase mapping (TPM)²⁴,²⁵,²⁷–²⁹ was performed in a single mid-ventricular short-axis slice using a 2D cine black-blood phase-contrast MRI sequence with velocity encoding in three directions. The TPM images were analyzed in MATLAB (Mathworks, Natick, MA) using previously built tools.¹⁹ For each mouse, peak velocities were calculated on a segmental level using AHA segmentation model and global peak systolic and diastolic velocity was calculated as the mean of the segmental peak velocities.

2.2.3 | T1 Mapping

T1 mapping was performed in a single mid-ventricular short-axis slice using a compressed-sensing (CS) accelerated cardio-respiratory triggered spiral Look-Locker pulse sequence.³⁰ Imaging parameters included: acceleration factor = 2, time between inversions = 7 s, slice thickness = 1 mm, number of spiral interleaves for full Nyquist sampling = 84, pixel size = 100 x 100 μm², flip angle = 3° and number of averages = 3. To further

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>MRI Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRI protocol</td>
<td>Scan Time</td>
</tr>
<tr>
<td>1. Localizer</td>
<td>10–15 min</td>
</tr>
<tr>
<td>2. Multi-Slice balanced steady-state free precession MRI in short-axis orientation</td>
<td>20 min</td>
</tr>
<tr>
<td>3. Single long axis cine DENSE MRI</td>
<td>10–12 min</td>
</tr>
<tr>
<td>The following sequences were all performed at the same mid-ventricular short-axis slice</td>
<td></td>
</tr>
<tr>
<td>4. Tissue Phase Mapping</td>
<td>7–8 min</td>
</tr>
<tr>
<td>5. Cine DENSE MRI</td>
<td>10–12 min</td>
</tr>
<tr>
<td>6. T2 Mapping</td>
<td>7 min</td>
</tr>
<tr>
<td>7. NativeT1 Mapping</td>
<td>7–8 min</td>
</tr>
<tr>
<td>8. Post-contrast T1 mapping</td>
<td>21–24 min</td>
</tr>
</tbody>
</table>
reduce the scan time to 7 min, a segmented acquisition was used where 3 spiral interleaves were acquired per cardiac cycle. Following the T1 mapping protocol from a previous study, T1 mapping was performed pre-contrast and three times after contrast starting at 6 min after contrast administration (scan time ~ 7 min/scan). Heart rate did not vary between the injections. Gadolinium (0.2 mmol/kg, total volume injected: 150 μL, timing of injections: 8 min apart) was diluted in saline solution and administered by three intra-peritoneal injections. The undersampled images were reconstructed using a CS algorithm called Block LOw-rank Sparsity with Motion-guidance (BLOSM). The reconstructed images were analyzed in MATLAB (Mathworks, Natick, MA). For data analysis, regions of interest (ROIs) were placed in the myocardium and the blood pool to generate signal intensity-time curves. T1 values were obtained by fitting the signal intensity-time curves to a three-parameter monoexponential curve. The reciprocal of T1 (R1 = 1/T1) was used to plot the myocardial R1 against the blood-pool R1. Multiple post-contrast T1 measurements were made to improve the accuracy of the fit. Partition coefficient was estimated as the slope of the linear fit and a global myocardial ECV was obtained by multiplying the partition coefficient by (1-Hematocrit).

2.2.4 | T2 Mapping

An accelerated cardio-respiratory triggered T2-prepared fast imaging sequence using 90° and 180° radiofrequency pulses in a segmented Malcom-Levitt (MLEV) phase cycling scheme was used to perform fast T2 mapping in a single mid-ventricular slice. The sequence was similar to the one used in a prior study. Rate 2 undersampling was performed to accelerate the image acquisition and BLOSM was used for image reconstruction. Imaging parameters included: TR = 3.3 ms, FOV = 25 x 25 mm², image resolution = 200 x 200 μm², averages = 2, slice thickness = 1 mm, TEs = 1.6, 9.6, 17.6, 25.6, 33.6, 41.6 ms, scan time = 7 min.

2.2.5 | DENSE MRI

Cine-DENSE MRI was acquired in a short-axis mid-ventricular slice and in a long axis slice. The cine DENSE images were analyzed using the DENSE analysis tool, which is a semi-automatic technique built in MATLAB (Mathworks, Natick, MA) to quantify global peak circumferential strain (Ecc) and longitudinal contour strain (Econ). Imaging parameters included: TE/TR = 0.7/7.1 ms, FOV = 25 x 25 mm², image resolution = 200 x 200 μm², flip angle = 170°, number of spiral interleaves = 36, in-plane/through-plane encoding frequency = 1/0.5 cyc/mm, displacement encoding method = Sim 3 pt (XY). Sim 3 pt (XY) refers to the simple 3 point encoding method for DENSE where one scan is the phase reference image and the other scans encode for displacement in the orthogonal (XY) directions.

2.3 | Histopathology

A subgroup of animals was sacrificed at weeks 5/6 following treatment initiation to evaluate histopathologic changes to the myocardium (Dox n = 4, DT n = 3). Details are in the Supplementary Information. Samples were collected from the mid LV for transmission electron microscopy (TEM).

For TEM, samples were reviewed for characteristic findings associated with cardiotoxicity including decrease in number of myocardial fibrils, mitochondrial distortion, nuclear degeneration, disorganized sarcoplasmic reticulum, and vacuoles in the sarcotubular system. For histopathology, samples were stained with hematoxylin and eosin (H and E) and terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay to assess for apoptosis. (See Supplementary Information for additional details).

2.4 | Statistical analysis

All data are presented as mean ± standard deviation. Differences between baseline and 5/6 and 10 week MRI parameters were compared using a linear mixed model analysis to account for repeated measures and changing group sizes due to euthanasia. The treatment group (Dox vs DT) was controlled for in the combined comparison by including this variable as a fixed factor in the model. A post-hoc Bonferroni correction was employed to account for multiple comparisons. A similar subgroup analysis was performed in the individual treatment groups (Dox and DT). Pearson correlation was performed between MRI parameters and changes in ejection fraction from baseline. Additional comparison between MRI parameters in mice with ejection fraction reduced by >5% (reduced EF) and preserved EF were performed using t-test. A p-value <0.05 was considered significant. Additionally, Two-tailed paired T-test was used to identify significant differences in TPM parameters over the timecourse of the study. P < 0.05 was considered significant. All findings were also analyzed as the total cohort (Dox and DT), as well as subgroup analysis in Dox and DT groups.
3 | RESULTS

3.1 | Weight and Hematocrit

There was overall increased body weight of mice over the course of the study which was statistically significant at 10 weeks (21.7 ± 1.6 g, median: 22 g, IQR: 2 g at baseline vs. 23.2 ± 1.3 g, median: 23 g, IQR: 2 g at 10 weeks, p = 0.03). This trend was similar in both subgroups and was also statistically significant in the DT group (p = 0.02). There was a decrease in hematocrit (HCT) over the course of the study which is a known effect of treatment with doxorubicin. The difference from baseline (49.7 ± 1.8%) was significant at both 5/6 weeks (47.1 ± 2.1%, p < 0.001) and 10 weeks (46.6 ± 1.6%, p < 0.01). These trends were similar in the D and DT subgroup analysis (Table 2).

3.2 | Cardiac MRI

Functional and tissue characterization parameters for cardiac MRI are summarized in Table 2.

3.2.1 | Tissue Characterization

Treatment with doxorubicin resulted in decreased myocardial native T1 at 5/6 weeks (Figure 2, 1,504 ± 214 ms vs 1,318 ± 169 ms, median: 1279 ms, IQR: 256 ms, p = 0.02) which normalized at 10 weeks (1,501 ± 156 ms, median: 1473 ms, IQR: 210 ms, p > 0.99). These trends were similar in two groups, although the difference at 5/6 weeks was not statistically significant in the DT subgroup. ECV decreased from baseline (34 ± 10%, median: 31%, IQR: 10%) over the course of the study (5/6 weeks: 27 ± 10%, median: 25%, IQR: 9%, p = 0.09; 10 weeks: 21 ± 8%, median: 16%, IQR: 14%, p = 0.01). This difference was most pronounced in the Dox subgroup (baseline: 37 ± 10%, median: 36%, IQR: 2.5% vs 10 week: 19 ± 7%, median: 16%, IQR: 12%, p = 0.03), although the DT subgroup exhibited similar non-significant trends. There were no statistically significant differences in T2 in the combined cohort or subgroup analysis.

3.2.2 | Functional Characterization

There were no statistically significant changes in ejection fraction (Figure 3A) in any of the subgroups at any of the timepoints. EF in total cohort was decreased from 68 ± 14%, median: 65%, IQR: 20% at baseline to 57 ± 10%, median: 61%, IQR: 17% at 10 weeks (p = 0.03).

In the total cohort, peak diastolic longitudinal velocity was reduced at 6 weeks by paired T-test (Table 2, −1.2 ± 0.3 cm/s, median: −1.2 cm/s, IQR: 0.5 cm/s at baseline vs. -1.0 ± 0.3 cm/s, median: −1.0 cm/s, IQR: 0.4 cm/s at 6 weeks, p < 0.05) while this normalized at 10 weeks. Additionally, peak systolic longitudinal velocity was reduced at 6 weeks and 10 weeks by paired T-test in the total cohort (0.8 ± 0.3 cm/s, median: 0.8 cm/s, IQR: 0.4 cm/s at baseline vs. 0.6 ± 0.2 cm/s, median: 0.6 cm/s, IQR: 0.3 cm/s at 10 weeks, p < 0.05). In the Dox group, peak systolic longitudinal velocity was reduced at 5/6 weeks by paired T-test (Figure 3B, 0.9 ± 0.3 cm/s, median: 0.8 cm/s, IQR: 0.4 cm/s at baseline vs. 0.6 ± 0.3 cm/s, median: 0.6 cm/s, IQR: 0.4 cm/s at 6 weeks, p < 0.05) while this normalized at 10 weeks. In the DT group, there were no significant differences in any of the TPM parameters over the entire study period. Figure 4 shows example TPM magnitude and phase difference images obtained from a mouse along with the systolic longitudinal velocity maps obtained in a mouse at different timepoints.

There were no significant differences in the DENSE-derived circumferential strain (Ecc) measurements at any of the post-treatment time points in the total cohort or the Dox group or DT group over the entire study period. There were no significant differences in longitudinal contour strain measurements (Econ) in any of the groups over the entire study period.

3.2.3 | Ejection fraction Subgroup Analysis

A total of 6/18 mice (2 Dox, 4 DT) exhibited >5% decrease in ejection fraction at 5/6 weeks and 6/11 mice (4 Dox, 2 DT) exhibited >5% decrease in ejection fraction at 10 weeks. There were no differences in MRI parameters at baseline, week 5/6, or 10 weeks in any MRI parameters between preserved and reduced EF groups. Baseline hematocrit (r = −0.56, p = 0.02) and ejection fraction (r = −0.57, p = 0.01) were inversely correlated with differences in ejection fraction at week 5/6 (Figure 5A), while baseline strain with DENSE was inversely correlated with differences in ejection fraction from baseline at 10 weeks (Figure 5B, −0.68, p = 0.04). No additional correlations were identified at baseline or later time points.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total Cohort</th>
<th>Doxorubicin</th>
<th>Doxorubicin &amp; Trastuzumab</th>
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</thead>
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<tr>
<td></td>
<td>Baseline (n = 18)</td>
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<td>10 wks (n = 11)</td>
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<tr>
<td>Hematocrit (%)</td>
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<td>47.1 ± 2.1*</td>
<td>46.6 ± 1.6**</td>
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<td>Heart Rate (bpm)</td>
<td>555 ± 49</td>
<td>578 ± 99</td>
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<td>Body weight (g)</td>
<td>21.7 ± 1.6</td>
<td>22.2 ± 1.5</td>
<td>23.2 ± 1.3**</td>
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<tr>
<td>Ejection Fraction (%)</td>
<td>68.4 ± 13.5</td>
<td>56.9 ± 10.2**</td>
<td>71.7 ± 10.7</td>
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<td>Native T1 (ms)</td>
<td>1.504 ± 214</td>
<td>1.501 ± 156*</td>
<td>1.588 ± 215</td>
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<tr>
<td>T2 (ms)</td>
<td>41 ± 7</td>
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<td>ECV</td>
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<td>0.21 ± 0.08**</td>
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</tr>
<tr>
<td>Peak radial velocity (cm/s)</td>
<td>Systole 1.1 ± 0.3</td>
<td>1.1 ± 0.2</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>Diastole −1.2 ± 0.3</td>
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<td>−1.2 ± 0.4</td>
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<tr>
<td>Peak longitudinal velocity (cm/s)</td>
<td>Systole 0.8 ± 0.3</td>
<td>0.6 ± 0.2**</td>
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<tr>
<td>Diastole −1.2 ± 0.3</td>
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<tr>
<td>Peak circumferential strain (%)</td>
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<td>Peak longitudinal contour strain (%)</td>
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<td>−0.10 ± 0.02</td>
<td>−0.10 ± 0.03</td>
</tr>
</tbody>
</table>

Bold p < 0.05.
*p < 0.05 vs. baseline,
**p < 0.05 vs. baseline,
#p < 0.05 vs. 6 wk.
In both the Dox and DT group, all sacrificed mice demonstrated minimal apoptosis (1–2 nuclei per HPF) on TUNEL staining which was the objective of our dosing strategy (Figure 6). There was no evidence of significant edema or fibrosis on Hematoxylin and eosin staining. There was no evidence of significant edema or fibrosis in any of the examined specimens. At electron microscopy, all examined specimens demonstrate changes which have previously been associated with cardiotoxicity including enlarged mitochondria with thickened cristae, vacuolization, and varying degrees of sarcomere disruption. Changes were similar in both the Dox and DT subgroups.

**FIGURE 3** Ejection fraction (A) and Peak systolic longitudinal velocity (B) in the entire cohort (Total), doxorubicin only (Dox), and doxorubicin + trastuzumab (DT) over the study period. *p < 0.05 and **p < 0.05 with respect to pre-treatment in the same group

### 3.3 | Histopathology and Ultrastructural Findings

In both the Dox and DT group, all sacrificed mice demonstrated minimal apoptosis (1–2 nuclei per HPF) on TUNEL staining which was the objective of our dosing strategy (Figure 6). There was no evidence of significant edema or fibrosis on Hematoxylin and eosin staining. There was no evidence of significant edema or fibrosis on hematoxylin and eosin staining in any of the examined specimens. At electron microscopy, all examined specimens demonstrate changes which have previously been associated with cardiotoxicity including enlarged mitochondria with thickened cristae, vacuolization, and varying degrees of sarcomere disruption. Changes were similar in both the Dox and DT subgroups.

### 4 | DISCUSSION

Our findings suggest that CMR myocardial functional and tissue characterization may identify myocardial dysfunction prior to apoptosis or onset of reduced LV systolic function in a mouse model of chemotherapy-induced cardiotoxicity. We found reduced myocardial velocities using TPM at 5/6 weeks following treatment with doxorubicin. We also found native T1 values were reduced at 5/6 weeks, but returned to near baseline levels at 10 weeks suggestive of a more acute phase response to treatment. Also, no changes were observed in myocardial T2 to indicate myocardial edema. Finally, we demonstrated that subcutaneous doxorubicin pellets can be used to generate cardiotoxicity in mice.
Multiple cellular mechanisms for chemotherapy-induced myocardial dysfunction have been demonstrated. Myocardial cell death (type I cardiotoxicity) results from treatment with doxorubicin, while other agents such as trastuzumab likely impact downstream myocyte repair (type II cardiotoxicity) which manifests at a later time point. It is interesting that there was little difference in either histopathologic or CMR findings between Dox and DT groups, perhaps suggesting longer term follow-up or higher dosing of trastuzumab or a different dosing regimen would be necessary to detect trastuzumab-related disease. In the case of doxorubicin, cardiomyocyte damage resulting from the creation of free radicals following the formation doxorubicin-iron complexes has been suggested as a possible mechanism. More recent studies have suggested that the unique gene expression in cardiac myocytes makes these cells especially sensitive to the mechanism that results in doxorubicin's cytotoxicity in cancer cells. Additional studies have suggested that doxorubicin impairs cellular energetics through mitochondrial damage and intra-
mitochondrial iron accumulation, as well as impairment of myocardial substrate utilization which can manifest as increased myocardial fatty acids. Ideally, diagnostic tests should focus on leveraging cellular level damage and functional changes to diagnose cardiomyopathy and CMR functional and tissue characterization is well-suited for this role.

While LVEF has been traditionally employed for cardiotoxicity diagnosis, recent work has questioned its utility as a means of categorizing cardiomyopathy. In our study, the reduction in myocardial velocity was a more sensitive indicator of cellular damage and impaired systolic function, whereas LVEF was not different from baseline in either treatment group. TPM has been shown to be highly reproducible with a low standard error, and in the context of our findings, should be useful in pre-clinical studies and clinical trials evaluating cardiotoxicity. Moreover, we found changes in myocardial velocity in a single mid-myocardial slice. The reduction in native T1 in the short interval following treatment with improvement at a later timepoint is in line with a recent clinical study that also demonstrated a similar finding in a cohort of sarcoma patients receiving doxorubicin treatment (cumulative dose of 360–400 mg/m² over 19 weeks). In this study, the authors reported that 48 hr post-treatment native T1 was significantly reduced in patients with a subsequent drop in LVEF, which normalized following the completion of chemotherapy. There are very few diseases that have a reduction in native T1 values, most notably intracellular glycosphingolipid accumulation in Fabry’s disease. There has been significant pre-clinical and clinical work performed on doxorubicin-treated subjects assessing changes in fatty acid metabolism. These studies demonstrate that reduced fatty acid metabolism and increased serum lipids convey resistance to cardiotoxic changes in models that reduce myocardial triacylglycerol. The reduction in ECV over the course of treatment may be due to increase in myocyte size. We found that LV mass increased during the course of treatment (data not shown). Thus, a relatively rapid cardiotoxicity CMR clinical protocol including TPM, T1 mapping, T2 mapping and cine SSFP images could be developed that provides extensive information on the state of the myocardium and could be performed throughout the course of treatment and follow-up period without exposure to gadolinium. In this study, we did not see any changes in T2 over the entire study period similar to a prior clinical study, however another animal study reported increased T2 reflective of myocardial edema post-chemotherapy. A prior study reported reduction in EF and increase in ECV in doxorubicin-treated mice at 10 weeks. The differences in results may be due to a higher sample size of doxorubicin-treated mice (N = 30° vs N = 9 in this study) or due to a different dosing regimen. With a higher sample size, it is also possible that we may see differences in CMR parameters in mice with reduced EF vs. mice with preserved EF and between the two treatments (doxorubicin only vs. combination therapy).

The current study is subject to several limitations. Most notably a relatively small sample size of animals, particularly at the 10 week time point limits statistical power. Another limitation of this study is that we have not performed phantom studies to validate the T2 values obtained using the quantitative T2 mapping sequence. The T2 values are on the higher side compared to the T2 values published previously in the literature. One reason for this could be B1 inhomogeneity. Additionally, more long-term follow-up studies are necessary to determine how early

**FIGURE 6** Histopathology and ultrastuctural findings from mice treated with doxorubicin alone (panel A) and doxorubicin + trastuzumab (panel B). The mouse in A had a stable ejection fraction and with native T1 decreased by 330 ms between scans. The mouse in B had an ejection fraction decrease of 7% and native T1 decreased by 140 ms between scans. Panels display (clockwise from top left of each panel) hematoxylin and eosin (H&E) staining at 20x magnification, electron microscopy at 4800x, electron microscopy at 9300x, and terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling (TUNEL) assay at 20x to assess for apoptosis. H&E stain demonstrate no significant edema or fibrosis in either animal. The TUNEL assay reveals no significant apoptosis (apoptotic nuclei stain brown with TUNEL). Electron microscopy demonstrates findings suggestive cardiotoxicity with vacuolization (white arrows), enlarged mitochondria with thickened cristae (*), and sarcomere damage (orange arrow). These findings are slightly more pronounced in the mouse in panel B.
imaging biomarkers correlate with long-term outcomes. Furthermore, we have not included a true control group in the current study, however we have compared all imaging endpoints to baseline CMR findings. Other studies have reported increased T2 (suggestive of myocardial edema), myocardial fibrosis and drop in ejection fraction in cardiotoxicity. We did not see similar changes which may be related to small sample size of animals, the dose or route of doxorubicin administration, and the time course of the study. It is encouraging that our histopathology and ultrastructural results also did not suggest interstitial fibrosis or edema, but did reveal ultrastructural findings associated with cardiotoxicity. Similarly, longitudinal strain has been suggested as a potential biomarker in cardiotoxicity.

In conclusion, our findings suggest myocardial functional and tissue characterization utilizing TPM and native T1 mapping may identify imaging biomarkers indicative of cardiac doxorubicin-induced cardiotoxicity prior to significant or irreversible myocardial injury or a reduction in left ventricular ejection fraction. Changes in these parameters may better reflect cellular and subcellular changes, which would not manifest as a reduction in global systolic function but may be important in identifying at-risk patients and initiating cardioprotective treatment regimens or changing the chemotherapy treatment plan to reduce the risk of irreversible myocardial damage. Future studies will look at the effect of cardioprotective strategies and examining the mechanisms underlying cardiotoxicity.

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE
All animal experiments are included as part of an Institutional Animal Care and Use Committee (IACUC) approved protocol.

CONSENT FOR PUBLICATION
Not applicable.

COMPETING INTERESTS
The authors declare that they have no competing interests.

FUNDING
This work was funded by RSNA Resident Grant (PI: Allen).

AVAILABILITY OF DATA AND MATERIALS
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

AUTHORS’ CONTRIBUTIONS
NKN designed the MRI sequences, the MRI protocol for the study and designed, collected, analyzed and interpreted all the MRI data collected in this study. NKN also drafted parts of the manuscript. SM was responsible for all the animal handling, doxorubicin injections, animal maintenance and animal sacrifice for histology studies. ZZ was responsible for histology studies. CY assisted with the MRI data collection. AR provided TPM post-processing code. NB was responsible for maintenance of the MR hardware and software to ensure smooth running of the study. FHE provided the DENSE sequence and post-processing code, T2 mapping sequence and the BLOSM image reconstruction code. JDC assisted with the study design, data interpretation and manuscript editing. MM provided the TPM sequence code and assisted with TPM data interpretation. DP is the director of the animal MRI facility and was responsible for running of MRI hardware, software, animal anesthesia, ECG gating and the smooth running of the animal study. JC, the chair of cardiac research was responsible for the study initiation, study design and data interpretation. BA was responsible for the study initiation, study design, performing the histology studies, data interpretation and drafting parts of the manuscript. The funding for the study also came from a grant awarded to BA. All authors were responsible for the manuscript editing.

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Additional supporting information may be found online in the Supporting Information section at the end of this article.