Introduction
Neoadjuvant chemotherapy is a well-established treatment option for patients with non-metastatic breast cancer. The patient response is correlated with survival. However, the optimal method for monitoring neoadjuvant therapy response has not been established. One factor that may affect the response of neoadjuvant therapy is the interstitial fluid pressure (IFP). Increased IFP prevents an effective delivery of therapeutic agents and reduces the efficacy of the therapy. Recently, subharmonic-aided pressure estimation (SHAPE) using contrast-enhanced ultrasound (CEUS) has been developed and its potential was demonstrated in animals as a non-invasive technique for IFP measurements. The SHAPE method estimates IFP based on the inverse relationship between the subharmonic signal magnitude from CEUS and IFP. The purpose of this study was to determine if 4D SHAPE can predict the response of breast cancer to neoadjuvant chemotherapy.

Materials and Methods
A. Subjects
Seventeen women with breast cancer (T1 or greater locally advanced breast cancer) and scheduled for neoadjuvant chemotherapy (approximately 16 therapy cycles over 3-6 months) participated in this study and all participants provided written informed consent.

B. Ultrasound examinations
Subjects underwent 4 US exams: immediately prior to therapy, at 10%, 60%, and 100% completion of neoadjuvant therapy. Ultrasound exams were performed using a modified Logiq 9 scanner with a 4010L probe (GE Healthcare, Milwaukee, WI, USA). Modified software enabled collection of radiofrequency (RF) data from a 4D pulse inversion subharmonic imaging mode (transmitting pulses at 5.8 MHz and receiving at 2.9 MHz). At each exam, 2D US baseline images were first acquired. Subjects then received a continuous IV infusion of 3 ml of Definity (Lantheus Medical Imaging, N Billerica, MA, USA) suspended in 50 ml saline via an antecubital vein, with infusion rates of 4 to 10 ml/min (titrated to effect). The acoustic output power used for SHAPE was optimized by running an automatic power control algorithm during the infusion. This power optimization was performed only for the initial exam and for the individual acoustic output power setting selected was then maintained through the rest of the US exams. After the optimization process, the infusion was halted for about 5 minutes to allow for clearance of bubbles. Then RF data was collected with no contrast agent in the 4D SHAPE mode. Infusion was restarted for collection of 3 sets of RF data with contrast agents. The volume rates for 4D data were 0.6 to 3.2 volume/second. Note, that the infusion interruption happened only for the initial exam in which the output power optimization was performed.

C. Evaluation of neoadjuvant therapy response
Clinical response was evaluated using the change in 3D tumor volume measurements from the US baseline images after the completion of neoadjuvant therapy. Subjects with more than 90% tumor volume reduction were rated as responders, while others were rated as partial/non-responders. This relatively coarse classification scheme was applied, due to the small sample size in this pilot study.

D. Data analysis
Using baseline images, the 3D tumor size change during therapy was compared to the clinical response and corresponding SHAPE results. RF data from 4D SHAPE exams were transferred to a PC for off-line analysis using Matlab (Mathworks, Natick, MA) and the subharmonic signal only from the contrast agent. Finally, the difference in the obtained subharmonic signal ratios from the tumor and the surrounding area was calculated as a relative estimate of IFP.

Results
A total of 17 subjects participated in the study (Fig 1). Ultrasound exams were performed in 3D modes and the tumor size change was observed through the therapy. Examples of 3D images from a subject prior to therapy and at the 10% completion of therapy are presented in Fig. 2. In this case, tumor size decreased after the therapy (from 4.2cm x 3.5cm x 3.4cm to 2.3 cm x 2.4 x 1.7). The symbol * represents significant differences with p < 0.05.

Conclusions
Monitoring neoadjuvant therapy at an early stage is challenging and the optimal method has not been established. In this pilot study, the 4D SHAPE method showed potential to monitor the clinical response of breast cancer to neoadjuvant chemotherapy as early as at 10% completion of therapy. As a functional assessment tool, 4D SHAPE may be useful to predict the neoadjuvant therapy response at an early stage.

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