Supplemental Online Content

Yu AF, Moore ZR, Moskowitz CS, et al. Association of circulating cardiomyocyte cell-free DNA with cancer therapy–related cardiac dysfunction in patients undergoing treatment for *ERBB2*-positive breast cancer. *JAMA Cardiol*. Published online May 31, 2023. doi:10.1001/jamacardio.2023.1229

This supplemental material has been provided by the authors to give readers additional information about their work.

eMethods

cfDNA extraction (12278, 12278_C, 12649, 12773, 12828)

cfDNA was extracted from 0.6-1 mL plasma using the MagMAX Cell-Free DNA Isolation Kit (ThermoFisher catalog # A29319) on the KingFisher Flex Purification System (ThermoFisher) according to the manufacturer's protocol. Samples were eluted in 45-58 µL elution solution.

Detection of unmethylated FAM101A cfDNA fragments by digital droplet PCR (12278_B, 12528, 12649_B, 12808, 12828_C)

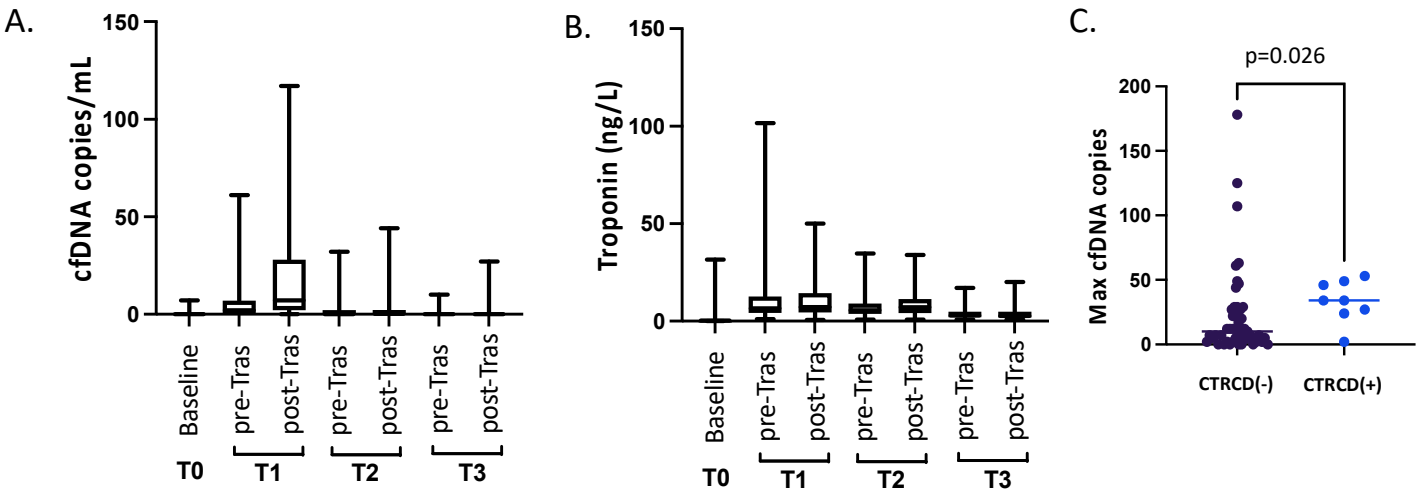
cfDNA underwent bisulfite conversion using the DNA Methylation-Gold kit (Zymo Research) following the manufacturer's instructions. Assays specific for the detection of unmethylated CpGs after bisulfite conversion at the *FAM101A* fragments were ordered through Bio-Rad.

Assay name	Forward sequence	Reverse sequence	Probe sequence	Fluorophore
FAM101A_ MethyFam	TATGGTTTGGTAATTTATTTAGAG	AAATACAAATCCCACAAATAAA	AATGTATGGTGAAATGTAGTGTTGGG	Fam
FAM101A_ MethyHex			AAAAATACTCAACTTCCATCTACAATT	Hex

Cycling conditions were tested to ensure optimal annealing/extension temperature as well as optimal separation of positive from empty droplets. Optimization was done with a known positive control, an ultramer spiked into gDNA.

After PicoGreen quantification, <0.1-9.0 ng bisulfite treated cfDNA were combined with locus-specific primers, FAM- and HEX-labeled probes, MseI, and digital PCR Supermix for probes (no dUTP). All reactions were performed on a QX200 ddPCR system (Bio-Rad catalog # 1864001) and each sample was evaluated in technical duplicates. Reactions were partitioned into a median of ~18,500 droplets per well using the QX200 droplet generator. Emulsified PCRs were run on a 96-well thermal cycler using cycling conditions identified during the optimization step (95°C 10'; 40 cycles of 94°C 30' and 55°C 1'; 98°C 10'; 4°C hold). Plates were read and analyzed with the QuantaSoft sotware to assess the number of droplets positive for FAM-tagged probes, HEX-tagged probes, both, or neither.

eFigure. Levels of cardiomyocyte cfDNA and high-sensitivity troponin I



A. Cardiomyocyte cfDNA at each timepoint over time in all patients. (B) HsTnI at each time point in all patients. Lines represent median value. C. Sum of cardiomyocyte cfDNA copies in both samples at T1 (pre + post trastuzumab) in patients with cancer therapy related cardiac dysfunction (CTRCD) compared to patients without CTRCD. Analysis includes 55 patients with both T1 plasma samples available.