Supplementary Material

The HER2 Inhibitor Lapatinib Potentiates Doxorubicin-Induced Cardiotoxicity through iNOS Signaling

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Key words: HER2 inhibitor, cardiotoxicity, doxorubicin, iPSC, iNOS

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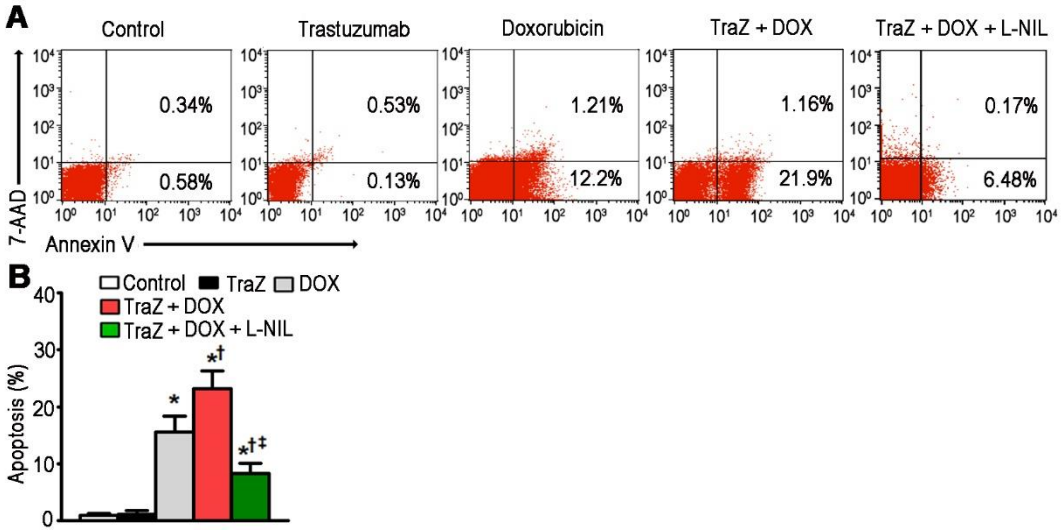
Supplementary method

Liquid chromatography/Tandem Mass Spectrometry analysis

The concentration of lapatinib in mouse plasma was determined by high-performance liquid chromatography (HPLC) with tandem mass spectrometry (LC-MS/MS) using validated methods. Plasma samples were extracted by protein precipitation using a solution of acetonitrile containing stable isotopically labeled letrozole-d4 (Sant Cruz) as an internal standard. After vortex-mixing for 3 minutes, the samples were centrifuged at approximately 14,000 rpm for 10 minutes. The extract was transferred into a 96 well plate for LC-MS/MS analysis by using an ultra-HPLC system (Agilent 1290) connected to a triple quadrupole tandem mass spectrometer (Agilent 6460) outfitted with an Agilent Jet Stream electrospray ionization source operating in the positive mode and multiple-reaction monitoring (MRM) detection with precursor product ion pairs of 581.2 → 365.2 for lapatinib, and 290.0 → 221.0 for the internal standard. Stock solution of letrozole-d4 was prepared in DMSO whereas lapatinib was prepared in methanol. Working stock solutions were diluted from the primary stock with dilution solution (methanol: water, 20:80, v: v) for fortification of the control plasma to prepare calibration standards. The calibration range was 10 to 10,000 ng/mL and was accomplished by weighted ($1/concentration^2$) linear regression of the ratio of the peak area of analyte to that of the added internal standard. All plasma samples were spiked with 10 µL of 500 ng/mL internal standard working solution. A reverse phase HPLC column was used for separation of the analyte. The analyte was eluted using a gradient of mobile phase A (1.0% formic acid in water) and mobile phase B (1.0% formic acid in methanol) from 35% to 95% mobile phase B.

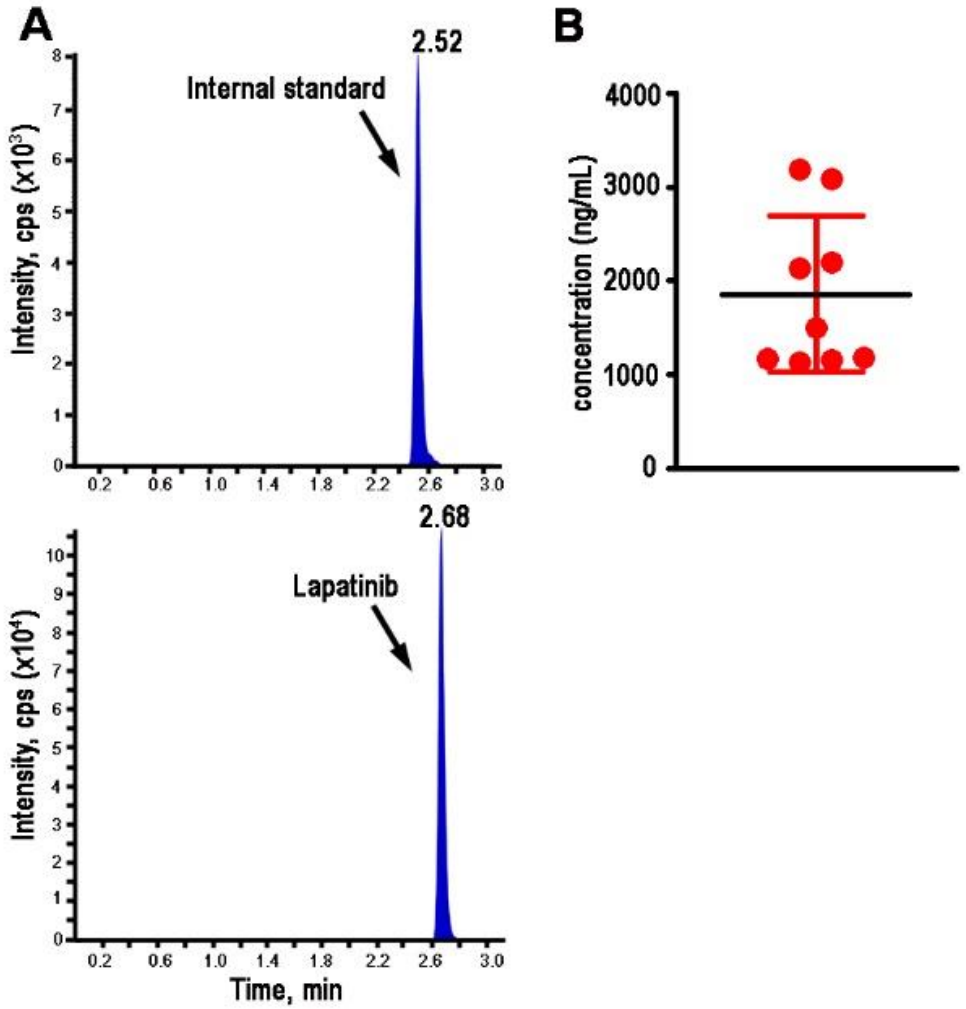
Supplementary Figures

Supplementary Figure 1



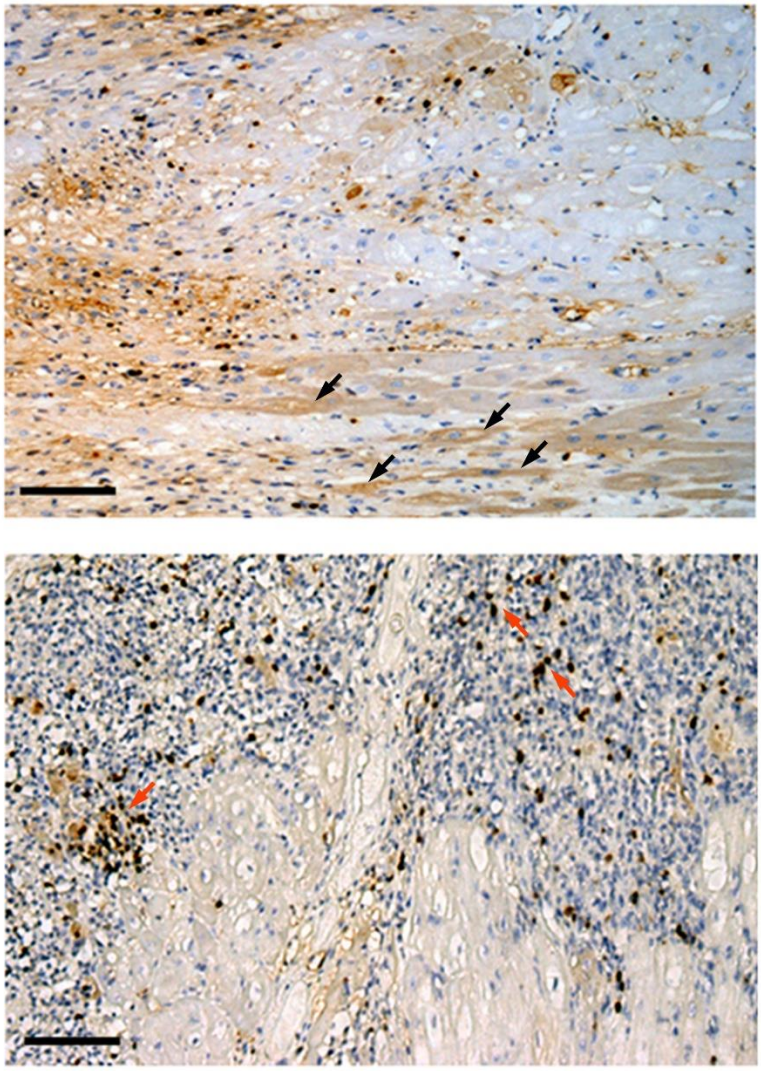
Supplementary Figure 1. N^6-(1-iminoethyl)-L-lysine (L-NIL) mediated inhibition of inducible nitric oxide synthase (iNOS) in hPSC-derived cardiomyocytes decreases TraZ-plus-DOX induced toxicity. hiPSC-CMs treated with vehicle (Control), TraZ alone (100 μg/mL), DOX alone (1.0 μmol/L), or both TraZ-plus-DOX, in the absence or presence of L-NIL (25 μmol/L).**A,** Proportion of viable, apoptotic and necrotic cells, based on flow cytometry analysis of 7-AAD and Annexin V co-labeling. **B,** Quantification of apoptosis upon L-NIL co-administration. All data are presented as mean ± SD ($n \geqq 3$). *$P < 0.05$ vs. control; † $P < 0.05$ vs. DOX; ‡ by $P < 0.05$ vs. TraZ + DOX. DOX = doxorubicin; TraZ = trastuzumab.

Supplementary Figure 2



Supplementary Figure 2. Plasma concentration of lapatinib after a single 10 mg/kg i.p. bolus in B6 mice. A, Representative mass ion chromatograms of a sample of lapatinib (3200 ng/mL) and internal standard. **B,** Preliminary studies in wild-type mice indicated that the maximum plasma concentration of lapatinib after a single 10 mg/kg i.p. injection ranged from approximately 1000 to 4000 ng/mL.

Supplementary Figure 3



Supplementary Figure 3. Immunohistochemical analysis of iNOS protein localization in LAP-plus-DOX-treated myocardium. The illustration shows iNOS staining positivity in cardiomyocytes (black arrows) and infiltrated mononuclear cells (red arrows). Scale bar indicates 100 μm.