Remote ischemic conditioning protects against anthracycline cardiotoxicity without impairing its antitumor activity

Short title: *RIC and anthracycline antitumoral effect*

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ABSTRACT

Anthracyclines remain a cornerstone of treatment for many cancer types; however, their cardiotoxic potential leads to cardiac dysfunction in a substantial proportion of patients, ultimately compromising long-term quality of life. Few strategies have proven effective in preventing anthracycline-induced cardiotoxicity (AIC). Among them, remote ischemic conditioning (RIC) has emerged as one of the most promising, having shown robust cardioprotective potential in preclinical studies and currently being evaluated in clinical trials. However, it remains unclear whether this intervention, while protecting the heart, could also inadvertently protect tumors from the cytotoxic effects of anthracyclines, thereby reducing their antitumor efficacy.

In this study, we investigated whether RIC protects against AIC in a tumor-bearing mouse model, allowing simultaneous assessment of both cardiac and tumoral responses. Cutaneous tumors were induced in CD1 mice using a DMBA/TPA protocol, followed by five weekly intraperitoneal injections of doxorubicin (5 mg/kg). Mice bearing tumors were randomized to receive doxorubicin alone or in combination with weekly RIC (three cycles of 5 min hindlimb ischemia/reperfusion). Longitudinal echocardiography was used to assess cardiac function, while tumor growth, survival, and body weight were monitored throughout the protocol.

Doxorubicin treatment reduced overall survival, inhibited tumor growth, and induced left ventricular systolic dysfunction and cardiac atrophy compared with untreated controls. RIC preserved left ventricular ejection fraction, partially attenuated early left ventricular atrophy, and showed a trend towards improved survival, without attenuating the antitumor efficacy of doxorubicin, as tumor suppression remained comparable between treatment groups. These findings demonstrate that RIC preserves cardiac systolic function during anthracycline chemotherapy in tumor-bearing mice without impairing the antitumor efficacy of the drug. The results support RIC as a simple, safe, and low-cost non-pharmacological strategy to mitigate AIC with potential translational relevance for oncology patients.

Keywords:

Anthracyclines, cancer, cardio-oncology, cardiotoxicity, doxorubicin, remote conditioning.

ABBREVIATIONS

AIC = anthracycline-induced cardiotoxicity

DMBA = 7,12-dimethylbenz[a]anthracene

DOX = doxorubicin

HF = heart failure

LV = left ventricle / left ventricular

LVEF = left ventricle ejection fraction

RIC = remote ischemic conditioning

TPA = 12-O-tetradecanoylphorbol-13-acetate

INTRODUCTION

Anthracyclines, administered alone or in combination, remain the cornerstone of therapy for many malignancies, with doxorubicin (DOX) widely used in both hematological and solid tumors. However, their intrinsic cardiotoxic potential represents a major non-cancer cause of morbidity and mortality among long-term cancer survivors. Registry data indicate that anthracycline-induced cardiotoxicity (AIC) is frequent and exerts a significant impact on outcomes, underscoring the urgent need for preventive approaches.[37] The *2022 ESC Guidelines on cardio-oncology* have consolidated this discipline, emphasizing structured risk stratification, longitudinal cardiac monitoring, and early intervention as central components of care.[39] Nevertheless, current pharmacological preventive cardioprotective options remain limited in both scope and efficacy, highlighting the need for safe, non-pharmacological strategies that can be implemented alongside standard.[27, 42]

Remote ischemic conditioning (RIC) is a non-invasive intervention that has been shown to be safe in multiple experimental studies in the context of myocardial ischemia-reperfusion injury and stroke, among other conditions.[24] In essence, RIC consists of brief episodes of ischemia followed by reperfusion in a remote organ (typically a limb), which triggers protective signaling that renders the heart more resistant to a subsequent injurious insult. Despite such encouraging preclinical data, RIC's performance in clinical trials of ischemia-reperfusion injury remains inconclusive [20, 22]. This interpretation is supported by several retrospective analyses and contemporary reviews demonstrating that neutral outcomes in major RIC trials likely reflect patient-related factors such as advanced age, comorbidities and polypharmacy, all of which can blunt endogenous cardioprotective signaling.[4, 12, 31, 32] Furthermore, it has been emphasized that several key clinical trials were conducted in settings with very low event rates in the control arm, thereby limiting statistical power to detect incremental benefit.[25] In addition, the intervention is most efficacious when initiated before the damage occurs and much less so when applied during an established injury.[16, 47] Accordingly, contexts such as acute myocardial infarction or stroke are imperfect for RIC because the timing of the insult is unpredictable. By contrast, the setting of anthracycline cardiotoxicity offers an ideal scenario: the administration of anthracyclines is scheduled and predictable, so RIC can be delivered in advance of the chemotherapeutic insult. This concept has been recently reinforced in a comprehensive review highlighting anthracycline cardiotoxicity as a highly suitable and mechanistically coherent framework for evaluating conditioning strategies, given its predictable timing and cumulative nature.[23] In a large-animal porcine model of AIC, application of RIC immediately before each doxorubicin administration significantly preserved left ventricular ejection fraction (LVEF) and mitigated mitochondrial damage and

fibrosis.[14] Based on these data, the ongoing RESILIENCE trial (N=600) is testing the benefit of RIC in high-risk patients undergoing anthracycline therapy.[43] In the smaller (N=55) ERIC-ONC preceding trial RIC did not affect the rise in biomarkers; however, that study was underpowered, used troponin release as a primary endpoint (which is debatable as the ideal endpoint in the context of AIC).[40] Of note, in the ERIC-ONC trial, patients allocated to RIC had a small increase in early cancer deaths, likely secondary to a disbalance in baseline characteristics, with greater proportion of patients with metastatic disease or relapse cancer randomized to the RIC.[40] Importantly, this theoretical concern has been explicitly raised before in the cardiovascular and cardio-oncology literature, where it has been suggested that the pleiotropic prosurvival signalling activated by RIC might also, in principle, confer protection to malignant cells during anthracycline therapy.[9, 14, 19, 26, 30] These prior publications underscore the need for experimental models capable of directly evaluating whether RIC modifies the antitumor efficacy of chemotherapy. Still, the possibility of RIC protecting the tumor itself cannot be completely ruled out based on these data. Thus, it remains crucial to test rigorously whether RIC provides cardioprotection in the AIC setting without diminishing the antitumor efficacy of these agents. There is not a single study in the literature testing the cardioprotection of RIC in the AIC setting in a tumor-bearing model.

MATERIALS & METHODS

Ethics

All animal procedures were reviewed and authorized by the *CNIC Institutional Animal Care and Ethics Committee* and the competent regional authority of Madrid (PROEX 099/16 and 176.3/20). Experiments were performed in full accordance with European legislation on animal research (*Directive 2010/63/EU and Recommendation 2007/526/EC*), transposed into Spanish law under *Royal Decree 53/2013*, and complied with the principles outlined in the *National Research Council's Guide for the Care and Use of Laboratory Animals*.

Mouse models

CD1 mice (4 weeks old, total *n* = 110) were obtained and housed under controlled environmental conditions (temperature 22 ± 2 °C, humidity 54%, 12:12 h light–dark cycle) with free access to water and standard chow. Animals were shaved on the dorsal surface using an electric clipper one week before carcinogenesis protocols (4 weeks old). CD1 mice are known for their susceptibility to developing skin tumors in response to 7,12-dimethylbenz[a]anthracene/12-O-tetradecanoylphorbol-13-acetate (DMBA DMBA/TPA treatment.[2, 51] Animals were distributed into three groups: Tumor CTRL (n = 5), Tumor + DOX (n = 75), and Tumor + DOX + RIC (n = 30) (**Figure 1A-B**). Based on our previous reports indicating early mortality in anthracycline-treated mice, the Tumor+DOX group was prospectively oversampled to compensate for expected attrition and ensure adequate power at later timepoints. RIC is not associated with increased mortality in preclinical AIC models; therefore, the Tumor+DOX+RIC group was not enlarged. All animals were randomized before treatment allocation. After completion of tumor induction, mice were randomly allocated to the Tumor+DOX or Tumor+DOX+RIC groups prior to the first chemotherapy dose.

Tumor induction

Cutaneous carcinogenesis was initiated in 4-weeks old mice by a single topical application of DMBA (1 mg/mL, 100 µL) to the shaved dorsal skin. CD1 mice at 4 weeks of age are in a peri-pubertal stage, which represents the standard developmental window for initiating DMBA/TPA carcinogenesis protocols. This age ensures efficient tumor initiation and reproducible papilloma formation, as consistently reported in the literature. DMBA functions as a polyaromatic hydrocarbon that is metabolized into highly reactive species capable of damaging DNA and generating mutations in epidermal cells. After a latency

period allowing initiated cells to acquire preneoplastic features, tumor promotion was induced with TPA (100 µM, 150 µL) applied twice weekly for 10 consecutive weeks. TPA is a tumor promoter acting as a phorbol ester receptor agonist, stimulating cell proliferation, inflammation, and angiogenesis, thereby driving tumor growth and progression. Animals were weighed weekly as a general safety measure, and skin tumor formation was recorded weekly throughout the study. Additional shaving was performed as required for accurate tumor assessment. Animals in all groups were closely monitored for signs of toxicity, including weight loss or mortality.

Anesthesia protocol

To minimize stress during topical applications, RIC, and photographic documentation, mice were anesthetized with sevoflurane delivered via a calibrated vaporizer connected to a closed induction chamber and nose cone on a temperature-controlled surgical bench. Anesthesia was induced at 4–5% sevoflurane in 1 L/min O_2 until loss of the righting reflex and maintained at 1.5–2.5% in 0.2–0.5 L/min O_2 for a total duration <5 min per procedure. Animals were continuously monitored for respiratory rate, depth of breathing, and pedal withdrawal reflex to ensure an appropriate anesthetic plane. Concentrations were adjusted as required to prevent movement without compromising vital signs. Following each session, sevoflurane was discontinued and animals recovered in 100% O_2 until full spontaneous mobility was observed. Weekly dorsal photographs were taken under standardized illumination, distance, and angle, with a millimeter scale included for accurate quantification.

Doxorubicin administration

Chemotherapy was delivered intraperitoneally at 5 mg/kg once per week for 5 consecutive weeks (cumulative dose: 25 mg/kg), as previously described.[10] This protocol is known to induce AIC with systolic dysfunction in CD-1 mice.[10] A commercially available formulation of doxorubicin (*Meiji Pharma, #999958.2*) was used for all injections. In groups receiving RIC, DOX administration was performed three days after each conditioning session to maintain a consistent temporal relationship between both interventions.

Remote ischemic conditioning

Following completion of the TPA schedule, mice in the DOX+RIC group underwent RIC once per week for five consecutive weeks. Each session consisted of three cycles of 5-min ischemia followed by 5-min reperfusion applied to the same hindlimb.

Procedures were performed under sevoflurane anesthesia as described above. RIC sessions were consistently scheduled three days prior to the corresponding DOX injection throughout the treatment course.

Tumor quantification

Skin tumors were monitored weekly under light sevoflurane anesthesia to ensure immobility and standardized imaging conditions. Photographs were collected from DMBA application (week −15) through 9 weeks post-DOX for all animals, generating several raw images. Representative images for each group and predefined timepoints are presented in Figure 4. Standardized digital photographs of the dorsal skin were acquired at fixed distance and illumination. Each image included a calibrated millimetre reference card bearing the individual animal identification number, which enabled accurate spatial scaling and minimized the risk of allocation errors. Quantitative analysis was performed using *Fiji/ImageJ software (NIH, Bethesda, MD, USA)*. The pixel-to-length ratio was calibrated for each image by setting the 84-mm reference on the scale card with the "Set Scale" function. Tumor burden was determined by manual delineation of individual lesions using the freehand selection tool. Each region of interest (ROI) was stored in RoiSet files for traceability and subsequent analysis. The following parameters were recorded for each lesion: tumor number, area, and perimeter. For longitudinal analysis, only lesions with a diameter ≥ 1 mm that persisted for at least two consecutive weeks were classified as papillomas and included in cumulative counts. Tumor assessment from serial photographs was performed by an investigator blinded to treatment allocation.

Echocardiography

Transthoracic echocardiography was performed by an experienced operator blinded to treatment allocation, using a high-resolution ultrasound platform (*Vevo 2100, VisualSonics, Toronto, Canada*) equipped with a 30-MHz linear array transducer. Imaging included two-dimensional (2D) and M-mode acquisitions at frame rates >230 frames/s. Mice were maintained under light anesthesia with 0.5–2% isoflurane in oxygen, titrated to preserve a stable heart rate within 450 ± 50 beats/min. Animals were placed supine on a heated stage to maintain body temperature, and pre-warmed ultrasound gel was applied to the thorax to optimize acoustic coupling. A base–apex electrocardiogram was continuously recorded throughout the examination. Digital echocardiographic data were exported for offline analysis using *Vevo 2100 Workstation software*. LV systolic function was quantified from standard parasternal long-axis (LAX) and short-axis (SAX) views in M-mode formats, with all functional indices derived from these recordings. Echocardiography studies were performed at baseline (post-TUMOR, pre-DOX; week −4), and

subsequently at 1 and 9 weeks after the completion of chemotherapy, corresponding to the early and late phases of anthracycline cardiotoxicity. All echocardiographic analyses were performed by an operator blinded to group allocation.

Statistics

All statistical analyses were performed using *GraphPad Prism software (version 9.5.1; GraphPad Software, San Diego, CA, USA)*. Results are presented as mean ± SD unless otherwise specified. The individual mouse was considered the unit of analysis in all experiments. For comparisons involving more than two groups, one-way or two-way analysis of variance (ANOVA) was applied, followed by appropriate post hoc multiple comparisons tests. Longitudinal data (e.g. body weight, tumor area, echocardiographic parameters) were analyzed using repeated-measures ANOVA. Survival data were evaluated by the log-rank (Mantel–Cox) test. A P value <0.05 was considered statistically significant.

RESULTS

Anthracycline regime was associated with increased mortality

Median survival analysis revealed that doxorubicin significantly reduced lifespan in tumor-bearing mice compared with tumor controls (see Kaplan–Meier curves in **Figure 2A-B**). RIC was associated with a non-significant trend (p=0.1434) towards increase survival compared to Tumor+DOX mice. Longitudinal monitoring of body weight demonstrated a progressive decline following doxorubicin administration, with comparable changes in Tumor+DOX and Tumor+DOX+RIC mice (**Figure 2C**).

Remote ischemic conditioning (RIC) prevents anthracycline-induced systolic dysfunction

At baseline (i.e. after tumor induction and immediately before doxorubicin regime initiation), LVEF was not different between the 3 study groups (Tumor CTRL, Tumor+DOX, and Tumor+DOX+RIC). One week after finishing the doxorubicin regime, LVEF showed a decline in Tumor+DOX group (p=0.0709 vs. Tumor CTRL), while it was not reduced in Tumor+DOX+RIC. At the end of the 9 weeks follow-up, LVEF in Tumor+DOX+RIC mice was higher than that in Tumor+DOX (p=0.0895 vs Tumor+DOX+RIC) although this difference did not reach statistical significance. LVEF in Tumor+DOX+RIC mice was not different from Tumor CTRL mice (i.e. not receiving anthracyclines). **Figure 3A** shows the trajectories of LVEF across the study duration.

Doxorubicin injections were associated with a significant LV atrophy, as shown by the reduced cardiac mass both in Tumor+DOX and in Tumor+DOX+RIC groups compared to Tumor CTRL group (**Figure 3B**). When analyzing the change in LV mass from baseline to 1 week (Δ LV mass), mice receiving RIC exhibited a smaller decline in mass than those treated with DOX alone, indicating a partial attenuation of early atrophy (**Figure 3C**). Representative images of echocardiograms are shown in **Figure 3D**. Full echocardiographic data is shown in **Table 1**.

Remote ischemic conditioning does not impair the anti-tumor efficacy of doxorubicin

Representative dorsal skin images illustrate tumor development during the carcinogenesis protocol and its evolution up to 9 weeks after completion of chemotherapy (**Figure 4A**). Quantification of total tumor area confirmed that doxorubicin treatment markedly suppressed tumor growth compared with mice not exposed to anthracyclines. This antitumor effect was observed across the entire follow-up in both groups receiving doxorubicin, with no differences between mice treated with doxorubicin

alone and those undergoing concomitant RIC (**Figure 4B**). The acute reduction in tumor burden observed one week after chemotherapy was equally evident in the RIC group, indicating that the addition of RIC did not alter the immediate antitumor effect of chemotherapy (**Figure 4C**).

DISCUSSION

In this study of anthracycline-induced cardiotoxicity (AIC) using a tumor-bearing mouse model, we found that (1) remote ischemic conditioning (RIC) prevents anthracycline-induced cardiac dysfunction, and (2) RIC does not impair the antitumor efficacy of anthracyclines. Serial quantification of cutaneous lesions demonstrated equivalent suppression of tumor burden in the Tumor+DOX and Tumor+DOX+RIC groups, both acutely and longitudinally. This finding carries an important translational implication, as any cardioprotective strategy in this context must ensure preservation of oncological efficacy. It has been speculated that, since RIC protects cardiac cells from the deleterious effects of anthracyclines, it might exert a similar protective influence on tumor cells. This theoretical concern has been explicitly raised before in the cardiovascular literature, where it has been argued that the prosurvival signaling pathways triggered by RIC might, in principle, also offer protection to malignant cells during anthracycline therapy.[19, 26] Our study directly addresses this concern through the use of a tumor-bearing model specifically designed to evaluate whether RIC modifies the antitumor efficacy of anthracyclines. In our model, the induced tumors are papillomas generated through the DMBA/TPA protocol, a validated and well-characterized model of skin carcinogenesis.[1, 2] This model represents a clinically analogous scenario of localized tumor, allowing a clear distinction between chemotherapy-related cardiac effects and oncological progression. Although it does not model metastatic or lethal cancer, it is ideally suited to address the specific safety question of whether RIC interferes with the antitumor efficacy of anthracyclines. This strengthens the validity of our approach by ensuring that differences in mortality or systemic tumor disease progression do not confound the assessment of cardiotoxicity.

Our results demonstrate that RIC preserved LVEF at both early and late time points compared with doxorubicin alone. This extends previous murine data showing that RIC attenuates adverse remodeling, fibrosis, and mortality following doxorubicin administration,[15] and is consistent with evidence that repeated RIC provides stronger and more durable protection.[17] Mechanistically, other experimental systems indicate that RIC activates prosurvival signaling cascades such as PI3K/Akt to mitigate anthracycline toxicity.[41] These protective effects have also been validated in large-animal models: in a clinically relevant porcine model, RIC preserved LVEF, reduced myocardial fibrosis, and maintained mitochondrial ultrastructure and function after repeated doxorubicin exposure mimicking the clinical scenario of chemotherapy regimes.[14] Collectively, these observations form a consistent cross-species body of evidence supporting the cardioprotective potential of RIC in the AIC setting. It is important to acknowledge, however, that the prototypic and historical endpoint of cardioprotection is infarct size

rather than contractile dysfunction. This distinction has been repeatedly emphasized in the cardioprotection field, particularly in the context of ischemia–reperfusion injury, where infarct size represents the gold-standard measure of efficacy.[5, 35] In the setting of anthracycline cardiotoxicity, contractile dysfunction remains the clinically relevant manifestation of injury, and therefore EF-based assessments are appropriate, yet they differ from the classic metrics used in conditioning research. Recognizing this distinction helps contextualize the interpretation of RIC effects across different cardiac injury paradigms. It should also be noted that some of the cardiac differences observed in our study reached only borderline statistical significance. These findings should therefore be interpreted with caution. Nonetheless, the direction and consistency of the effect across serial assessments support the overall conclusion that RIC attenuated DOX-induced cardiac dysfunction.

Mechanistically, it is relevant to acknowledge that STAT3 is a key mediator of the cardioprotective signaling activated by RIC, as demonstrated in both murine and translational models.[36, 46] STAT3 activation has also been proposed as a potential mechanism through which RIC could theoretically influence tumor biology or interfere with the antitumor effects of anthracyclines,[26] and recent reviews have highlighted that several intracellular cardioprotective pathways—including STAT3 and mitochondrial TERT—may overlap with signaling mechanisms relevant to cancer cell survival.[3, 11, 28-30, 33] These pathways represent an important area for future research; however, the primary objective of the present study was deliberately translational rather than mechanistic, aiming to address the specific and clinically relevant safety question of whether RIC might compromise the antitumor efficacy of anthracyclines. Our tumor-bearing model directly evaluates this concern, and our findings show no evidence of reduced antitumor efficacy when RIC is combined with doxorubicin.

In our study, doxorubicin induced a marked reduction in LV mass, consistent with cardiomyocyte atrophy as a key deleterious component of AIC. Analysis of Δ LV mass at 1 week post-DOX showed that RIC partially attenuated this early loss of myocardial mass compared with DOX alone. This observation aligns with mechanistic data showing that doxorubicin activates p38-MAPK and regulates the ubiquitin ligase atrogin-1, leading to cardiomyocyte atrophy.[50] More recently, cardiomyocyte atrophy has been recognized as an underestimated component of anthracycline cardiotoxicity.[7] Taken together, these findings suggest that while RIC preserves contractile function and partially limits early myocardial mass loss, it does not fully prevent structural remodeling, pointing to a dissociation between functional reserve and structural integrity, and highlighting atrophy as a distinct therapeutic target for future interventions. These results open the venue for cardioprotective strategies against anthracycline-induced cardiac atrophy that could be synergistic with RIC.[10]

The present findings complement the growing recognition that cardio-oncology must prioritize proactive prevention rather than reactive treatment.[53] As emphasized elsewhere, cardiotoxicity remains a leading non-cancer cause of morbidity and mortality among cancer survivors.[6, 44, 48, 52] While pharmacological cardioprotective agents such as dexrazoxane, beta-blockers, statins, and SGLT2 inhibitors have yielded mixed outcomes,[42] non-pharmacological strategies like RIC offer an appealing complementary approach. Previous works have underscored those at highest risk (i.e. those with known risk factors increasing the risk of AIC, including those with high ICOS-HF score ([38, 45, 49]) represent ideal populations for preventive.[18, 27]

Clinical data in the context of the benefits of RIC against AIC remain limited to small underpowered studies. In a randomized trial of pediatric cancer patients, RIC proved feasible and safe but did not attenuate biomarker release or early ventricular dysfunction.[8] A parallel adult study (ERIC-ONC) also yielded neutral results regarding troponin and cMyC release.[40] It is important to remark that patients included in both trials were considered at low risk for AIC. In that context, any intervention (including RIC) has low chances to show clinically meaningful benefits.[18, 27] In that sense, the ongoing RESILIENCE trial is testing the benefits of RIC in patients at high baseline risk for AIC.[43] Furthermore, these 2 small trials[8, 40] used a controversial surrogate outcome (release of cardiac troponin) in this specific context. Cardiac dysfunction in AIC is characterized by dysfunctional cardiomyocytes and not by an important early cell loss. The value of biomarkers reflecting cell loss in this context is questionable. To overcome this limitation, in the present study, the primary outcome was an imaging one and not biomarkers release. In the same line, the ongoing RESILIENCE trial uses a comprehensive cardiac magnetic resonance (CMR) imaging protocol as the primary outcome measure.[43]

The most important finding of the present study is that the cardioprotection afforded by RIC was not associated with any reduction in the efficacy of the antitumor effect of anthracyclines. This is especially relevant since in one of the small studies testing RIC in adult cancer patients, cancer-related mortality was greater in patients undergoing this intervention compared to controls.[40] While the most plausible reason for this increased cancer-related mortality was a baseline imbalance in the severity of cancer (i.e. those allocated to RIC had a greater cancer extension and had higher cases of relapse cancer), it raised a flag as to the possibility that RIC might have protected the cancer against chemotherapy. The present findings have clearly shown that RIC does not interfere with the antitumor effect of anthracyclines, and this is very reassuring in the context of ongoing trials, such as RESILIENCE,[43] and in view of a possible future implementation of this intervention in daily practice.

In conclusion, this preclinical study in tumor-bearing mice demonstrates that RIC preserves cardiac function after anthracycline chemotherapy without compromising its antitumor efficacy. By bridging experimental models and early human data, our work reinforces the rationale for ongoing clinical translation, exemplified by the RESILIENCE trial,[43] and supports RIC as a feasible, safe, and potentially impactful preventive strategy in cardio-oncology. Nonetheless, RIC did not completely prevented cardiac atrophy, an increasingly recognized therapeutic target in anthracycline cardiotoxicity.[7, 10] This opens venues for searching complementary cardioprotective strategies that specifically target atrophy pathways and could act synergistically with RIC.

From a translational standpoint, our findings underscore that non-pharmacological interventions such as RIC can preserve contractile performance without compromising antitumor efficacy. Future directions should incorporate advanced CMR tissue characterization alongside echocardiography, extend follow-up to capture survival and HF events, and evaluate combinatorial approaches that target atrophy pathways in addition to preserving systolic function. Ultimately, integrating pragmatic protective strategies like RIC into multidisciplinary cardio-oncology care will require harmonization with pharmacological interventions, rigorous clinical trial endpoints, and careful patient selection in ongoing initiatives.[43]

The strengths and limitations of our study frame the path forward. Key strengths include: (i) a tumor-bearing model that simultaneously quantifies both cancer and cardiac outcomes, thereby addressing the central concern of safety in cardio-oncology; (ii) a course-based RIC protocol temporally aligned with chemotherapy cycles, mimicking the clinical setting; and (iii) longitudinal imaging with echocardiography to capture functional trajectories over time. Together, these features provide a translationally relevant framework that bridges preclinical and clinical domains.

Limitations should also be acknowledged. First, experiments were restricted to a single species and one tumor model; additional validation in other models, including metastatic or hematological cancers, would confirm generalizability. Second, the absence of mechanistic dissection was intentional, as our focus was translational, but this precludes insights into the molecular determinants of RIC protection (and its divergences with the cancer interplay with anthracyclines). Third, we did not assess the coronary microcirculation, which is increasingly recognized as a relevant target of anthracycline toxicity and contributes to the progression of heart failure.[13, 21] Importantly, several studies have shown that RIC can confer protection at the microvascular level.[29, 34] The absence of microcirculatory assessment in our study precludes conclusions regarding the impact of RIC on this specific compartment and should be considered when interpreting our findings. Fourth, survival in

this model must be interpreted with caution, as DMBA/TPA papillomas do not cause cancer-related mortality. Accordingly, survival reflects treatment-related toxicity rather than oncological progression, and should be viewed as a safety indicator rather than a measure of antitumor efficacy.

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AUTHOR CONTRIBUTIONS

A. Díaz-Guerra performed the experiments, analyzed data, prepared the figures and wrote the manuscript. A. Clemente-Moragón supported data interpretation. Á. Pollán and L. López-Palomar assisted with the animal model. L. Cádiz supervised and revised the draft. B. Ibáñez conceived and led the study, reviewed and approved the manuscript.

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DECLARATIONS

Conflict of interest: The authors have reported that they have no relationships relevant to the contents of this paper to disclose.

REFERENCES

1. Abbotto E, Miro C, Piacente F, Salis A, Murolo M, Nappi A, Millo E, Russo E, Cichero E, Sturla L, Del Rio A, De Flora A, Nencioni A, Dentice M, Bruzzone S (2023) SIRT6 pharmacological inhibition delays skin cancer progression in the squamous cell carcinoma. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 166:115326 doi:10.1016/j.biopha.2023.115326
2. Abel EL, Angel JM, Kiguchi K, DiGiovanni J (2009) Multi-stage chemical carcinogenesis in mouse skin: fundamentals and applications. Nat Protoc 4:1350-1362 doi:10.1038/nprot.2009.120
3. Ale-Agha N, Jakobs P, Goy C, Zurek M, Rosen J, Dyballa-Rukes N, Metzger S, Greulich J, von Ameln F, Eckermann O, Unfried K, Brack F, Grandoch M, Thielmann M, Kamler M, Gedik N, Kleinbongard P, Heinen A, Heusch G, Godecke A, Altschmied J, Haendeler J (2021) Mitochondrial Telomerase Reverse Transcriptase Protects From Myocardial Ischemia/Reperfusion Injury by Improving Complex I Composition and Function. Circulation 144:1876-1890 doi:10.1161/CIRCULATIONAHA.120.051923
4. Bell RM, Basalay M, Botker HE, Beikoghli Kalkhoran S, Carr RD, Cunningham J, Davidson SM, England TJ, Giesz S, Ghosh AK, Golforoush P, Gourine AV, Hausenloy DJ, Heusch G, Ibanez B, Kleinbongard P, Lecour S, Lukhna K, Ntsekhe M, Ovize M, Salama AD, Vilahur G, Walker JM, Yellon DM (2022) Remote ischaemic conditioning: defining critical criteria for success-report from the 11th Hatter Cardiovascular Workshop. Basic Res Cardiol 117:39 doi:10.1007/s00395-022-00947-2
5. Botker HE, Hausenloy D, Andreadou I, Antonucci S, Boengler K, Davidson SM, Deshwal S, Devaux Y, Di Lisa F, Di Sante M, Efentakis P, Femmino S, Garcia-Dorado D, Giricz Z, Ibanez B, Iliodromitis E, Kaludercic N, Kleinbongard P, Neuhauser M, Ovize M, Pagliaro P, Rahbek-Schmidt M, Ruiz-Meana M, Schluter KD, Schulz R, Skyschally A, Wilder C, Yellon DM, Ferdinandy P, Heusch G (2018) Practical guidelines for rigor and reproducibility in preclinical and clinical studies on cardioprotection. Basic Res Cardiol 113:39 doi:10.1007/s00395-018-0696-8
6. Chang HM, Okwuosa TM, Scarabelli T, Moudgil R, Yeh ETH (2017) Cardiovascular Complications of Cancer Therapy: Best Practices in Diagnosis, Prevention, and Management: Part 2. J Am Coll Cardiol 70:2552-2565 doi:10.1016/j.jacc.2017.09.1095
7. Chen DS, Yan J, Yang PZ (2022) Cardiomyocyte Atrophy, an Underestimated Contributor in Doxorubicin-Induced Cardiotoxicity. Front Cardiovasc Med 9:812578 doi:10.3389/fcvm.2022.812578
8. Cheung YF, Li VW, So EK, Cheng FW, Yau JP, Chiu SY, Wong WH, Cheuk DK (2023) Remote Ischemic Conditioning in Pediatric Cancer Patients Receiving Anthracycline Chemotherapy: A Sham-Controlled Single-Blind Randomized Trial. JACC CardioOncol 5:332-342 doi:10.1016/j.jaccao.2022.11.020
9. Davidson SM, Arjun S, Basalay MV, Bell RM, Bromage DI, Botker HE, Carr RD, Cunningham J, Ghosh AK, Heusch G, Ibanez B, Kleinbongard P, Lecour S, Maddock H, Ovize M, Walker M, Wiart M, Yellon DM (2018) The 10th Biennial Hatter Cardiovascular Institute workshop: cellular protection-evaluating new directions in the setting of myocardial infarction, ischaemic stroke, and cardio-oncology. Basic Res Cardiol 113:43 doi:10.1007/s00395-018-0704-z
10. Diaz-Guerra A, Villena-Gutierrez R, Clemente-Moragon A, Gomez M, Oliver E, Fernandez-Tocino M, Galan-Arriola C, Cadiz L, Ibanez B (2024) Anthracycline Cardiotoxicity Induces Progressive Changes in Myocardial Metabolism and Mitochondrial Quality Control: Novel Therapeutic Target. JACC CardioOncol 6:217-232 doi:10.1016/j.jaccao.2024.02.005

11. Efentakis P, Andreadou I, Iliodromitis KE, Triposkiadis F, Ferdinandy P, Schulz R, Iliodromitis EK (2022) Myocardial Protection and Current Cancer Therapy: Two Opposite Targets with Inevitable Cost. Int J Mol Sci 23 doi:10.3390/ijms232214121
12. Ferdinandy P, Andreadou I, Baxter GF, Botker HE, Davidson SM, Dobrev D, Gersh BJ, Heusch G, Lecour S, Ruiz-Meana M, Zuurbier CJ, Hausenloy DJ, Schulz R (2023) Interaction of Cardiovascular Nonmodifiable Risk Factors, Comorbidities and Comedications With Ischemia/Reperfusion Injury and Cardioprotection by Pharmacological Treatments and Ischemic Conditioning. Pharmacological reviews 75:159-216 doi:10.1124/pharmrev.121.000348
13. Galan-Arriola C, Vilchez-Tschischke JP, Lobo M, Lopez GJ, de Molina-Iracheta A, Perez-Martinez C, Villena-Gutierrez R, Macias A, Diaz-Rengifo IA, Oliver E, Fuster V, Sanchez-Gonzalez J, Ibanez B (2021) Coronary microcirculation damage in anthracycline cardiotoxicity. Cardiovasc Res doi:10.1093/cvr/cvab053
14. Galan-Arriola C, Villena-Gutierrez R, Higuero-Verdejo MI, Diaz-Rengifo IA, Pizarro G, Lopez GJ, Molina-Iracheta A, Perez-Martinez C, Garcia RD, Gonzalez-Calle D, Lobo M, Sanchez PL, Oliver E, Cordoba R, Fuster V, Sanchez-Gonzalez J, Ibanez B (2021) Remote ischaemic preconditioning ameliorates anthracycline-induced cardiotoxicity and preserves mitochondrial integrity. Cardiovasc Res 117:1132-1143 doi:10.1093/cvr/cvaa181
15. Gertz ZM, Cain C, Kraskauskas D, Devarakonda T, Mauro AG, Thompson J, Samidurai A, Chen Q, Gordon SW, Lesnefsky EJ, Das A, Salloum FN (2019) Remote Ischemic Pre-Conditioning Attenuates Adverse Cardiac Remodeling and Mortality Following Doxorubicin Administration in Mice. JACC CardioOncol 1:221-234 doi:10.1016/j.jaccao.2019.11.004
16. Hausenloy DJ, Kharbanda RK, Moller UK, Ramlall M, Aaroe J, Butler R, Bulluck H, Clayton T, Dana A, Dodd M, Engstrom T, Evans R, Lassen JF, Christensen EF, Garcia-Ruiz JM, Gorog DA, Hjort J, Houghton RF, Ibanez B, Knight R, Lippert FK, Lonborg JT, Maeng M, Milasinovic D, More R, Nicholas JM, Jensen LO, Perkins A, Radovanovic N, Rakhit RD, Ravkilde J, Ryding AD, Schmidt MR, Riddervold IS, Sorensen HT, Stankovic G, Varma M, Webb I, Terkelsen CJ, Greenwood JP, Yellon DM, Botker HE, Investigators C-E-P (2019) Effect of remote ischaemic conditioning on clinical outcomes in patients with acute myocardial infarction (CONDI-2/ERIC-PPCI): a single-blind randomised controlled trial. Lancet 394:1415-1424 doi:10.1016/S0140-6736(19)32039-2
17. He Q, Wang F, Ryan TD, Chalasani M, Redington AN (2020) Repeated Remote Ischemic Conditioning Reduces Doxorubicin-Induced Cardiotoxicity. JACC CardioOncol 2:41-52 doi:10.1016/j.jaccao.2020.01.005
18. Henriksen PA, Rankin S, Lang NN (2023) Cardioprotection in Patients at High Risk of Anthracycline-Induced Cardiotoxicity: JACC: CardioOncology Primer. JACC CardioOncol 5:292-297 doi:10.1016/j.jaccao.2023.05.004
19. Heusch G (2023) Cardioprotection in cardio-oncology: a case for concern? Cardiovasc Res 119:e144-e145 doi:10.1093/cvr/cvad111
20. Heusch G (2013) Cardioprotection: chances and challenges of its translation to the clinic. Lancet 381:166-175 doi:doi: 10.1016/S0140-6736(12)60916-7
21. Heusch G (2022) Coronary blood flow in heart failure: cause, consequence and bystander. Basic Res Cardiol 117:1 doi:10.1007/s00395-022-00909-8
22. Heusch G (2020) Myocardial ischaemia-reperfusion injury and cardioprotection in perspective. Nature reviews 17:773-789 doi:10.1038/s41569-020-0403-y
23. Heusch G, Andreadou I, Bell R, Bertero E, Botker HE, Davidson SM, Downey J, Eaton P, Ferdinandy P, Gersh BJ, Giacca M, Hausenloy DJ, Ibanez B, Krieg T, Maack C, Schulz R, Sellke F, Shah AM, Thiele H, Yellon DM, Di Lisa F (2023) Health position paper and redox perspectives

on reactive oxygen species as signals and targets of cardioprotection. Redox Biol 67:102894 doi:10.1016/j.redox.2023.102894

24. Heusch G, Botker HE, Przyklenk K, Redington A, Yellon D (2015) Remote Ischemic Conditioning. J Am Coll Cardiol 65:177-195 doi:10.1016/j.jacc.2014.10.031
25. Heusch G, Gersh BJ (2020) Is Cardioprotection Salvageable? Circulation 141:415-417 doi:10.1161/CIRCULATIONAHA.119.044176
26. Heusch G, Rassaf T (2021) Protection from cardiotoxicity of cancer chemotherapy: a novel target for remote ischaemic conditioning? Cardiovasc Res 117:985-986 doi:10.1093/cvr/cvaa199
27. Ibanez B, Gomes-Silva M (2023) Remote Ischemic Conditioning for Anthracycline Cardiotoxicity: The Need to Protect the Most Vulnerable. JACC CardioOncol 5:356-359 doi:10.1016/j.jaccao.2023.05.002
28. Kleinbongard P (2023) Perspective: mitochondrial STAT3 in cardioprotection. Basic Res Cardiol 118:32 doi:10.1007/s00395-023-01003-3
29. Kleinbongard P, Amanakis G, Skyschally A, Heusch G (2018) Reflection of Cardioprotection by Remote Ischemic Perconditioning in Attenuated ST-Segment Elevation During Ongoing Coronary Occlusion in Pigs: Evidence for Cardioprotection From Ischemic Injury. Circ Res 122:1102-1108 doi:10.1161/CIRCRESAHA.118.312784
30. Kleinbongard P, Andreadou I (2024) Is There a Mitochondrial Protection via Remote Ischemic Conditioning in Settings of Anticancer Therapy Cardiotoxicity? Curr Heart Fail Rep doi:10.1007/s11897-024-00658-w
31. Kleinbongard P, Botker HE, Ovize M, Hausenloy DJ, Heusch G (2020) Co-morbidities and co-medications as confounders of cardioprotection-Does it matter in the clinical setting? Br J Pharmacol 177:5252-5269 doi:10.1111/bph.14839
32. Kleinbongard P, Neuhauser M, Thielmann M, Kottenberg E, Peters J, Jakob H, Heusch G (2016) Confounders of Cardioprotection by Remote Ischemic Preconditioning in Patients Undergoing Coronary Artery Bypass Grafting. Cardiology 133:128-133 doi:10.1159/000441216
33. Kleinbongard P, Skyschally A, Gent S, Pesch M, Heusch G (2018) STAT3 as a common signal of ischemic conditioning: a lesson on "rigor and reproducibility" in preclinical studies on cardioprotection. Basic Res Cardiol 113:3 doi:10.1007/s00395-017-0660-z
34. Lau JK, Roy P, Javadzadegan A, Moshfegh A, Fearon WF, Ng M, Lowe H, Brieger D, Kritharides L, Yong AS (2018) Remote Ischemic Preconditioning Acutely Improves Coronary Microcirculatory Function. Journal of the American Heart Association 7:e009058 doi:10.1161/JAHA.118.009058
35. Lecour S, Andreadou I, Botker HE, Davidson SM, Heusch G, Ruiz-Meana M, Schulz R, Zuurbier CJ, Ferdinandy P, Hausenloy DJ, on behalf of the European Union CCAC (2021) IMproving Preclinical Assessment of Cardioprotective Therapies (IMPACT) criteria: guidelines of the EU-CARDIOPROTECTION COST Action. Basic Res Cardiol 116:52 doi:10.1007/s00395-021-00893-5
36. Lieder HR, Kleinbongard P, Skyschally A, Hagelschuer H, Chilian WM, Heusch G (2018) Vago-Splenic Axis in Signal Transduction of Remote Ischemic Preconditioning in Pigs and Rats. Circ Res 123:1152-1163 doi:10.1161/CIRCRESAHA.118.313859
37. Lopez-Sendon J, Alvarez-Ortega C, Zamora Aunon P, Buno Soto A, Lyon AR, Farmakis D, Cardinale D, Canales Albendea M, Feliu Batlle J, Rodriguez Rodriguez I, Rodriguez Fraga O, Albaladejo A, Mediavilla G, Gonzalez-Juanatey JR, Martinez Monzonis A, Gomez Prieto P, Gonzalez-Costello J, Serrano Antolin JM, Cadenas Chamorro R, Lopez Fernandez T (2020) Classification, prevalence, and outcomes of anticancer therapy-induced cardiotoxicity: the CARDIOTOX registry. Eur Heart J 41:1720-1729 doi:10.1093/eurheartj/ehaa006

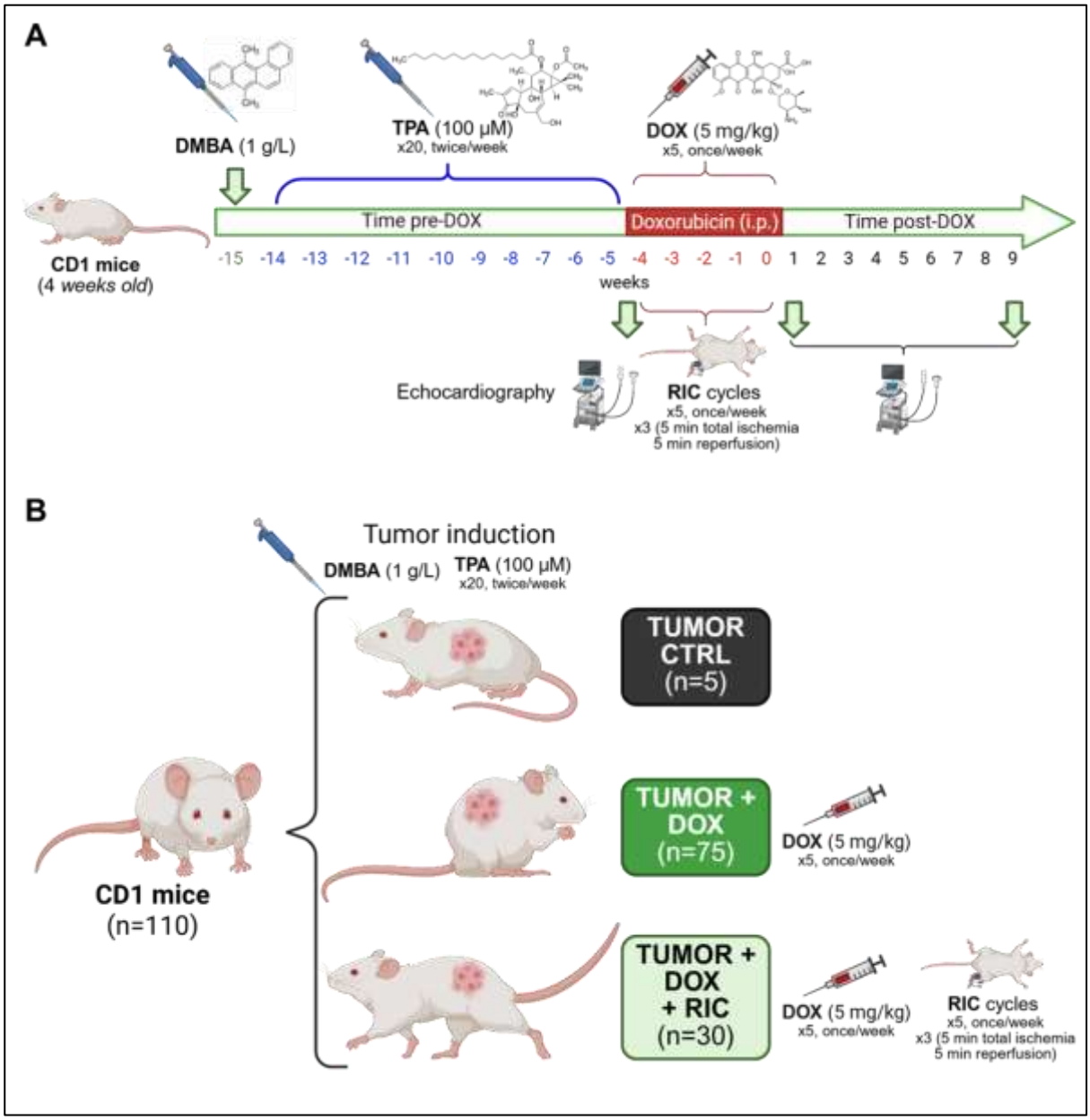
38. Lyon AR, Dent S, Stanway S, Earl H, Brezden-Masley C, Cohen-Solal A, Tocchetti CG, Moslehi JJ, Groarke JD, Bergler-Klein J, Khoo V, Tan LL, Anker MS, von Haehling S, Maack C, Pudil R, Barac A, Thavendiranathan P, Ky B, Neilan TG, Belenkov Y, Rosen SD, Iakobishvili Z, Sverdlov AL, Hajjar LA, Macedo AVS, Manisty C, Ciardiello F, Farmakis D, de Boer RA, Skouri H, Suter TM, Cardinale D, Witteles RM, Fradley MG, Herrmann J, Cornell RF, Wechelaker A, Mauro MJ, Milojkovic D, de Lavallade H, Ruschitzka F, Coats AJS, Seferovic PM, Chioncel O, Thum T, Bauersachs J, Andres MS, Wright DJ, Lopez-Fernandez T, Plummer C, Lenihan D (2020) Baseline cardiovascular risk assessment in cancer patients scheduled to receive cardiotoxic cancer therapies: a position statement and new risk assessment tools from the Cardio-Oncology Study Group of the Heart Failure Association of the European Society of Cardiology in collaboration with the International Cardio-Oncology Society. Eur J Heart Fail 22:1945-1960 doi:10.1002/ejhf.1920
39. Lyon AR, Lopez-Fernandez T, Couch LS, Asteggiano R, Aznar MC, Bergler-Klein J, Boriani G, Cardinale D, Cordoba R, Cosyns B, Cutter DJ, de Azambuja E, de Boer RA, Dent SF, Farmakis D, Gevaert SA, Gorog DA, Herrmann J, Lenihan D, Moslehi J, Moura B, Salinger SS, Stephens R, Suter TM, Szmit S, Tamargo J, Thavendiranathan P, Tocchetti CG, van der Meer P, van der Pal HJH, Group ESCSD (2022) 2022 ESC Guidelines on cardio-oncology developed in collaboration with the European Hematology Association (EHA), the European Society for Therapeutic Radiology and Oncology (ESTRO) and the International Cardio-Oncology Society (IC-OS). Eur Heart J 43:4229-4361 doi:10.1093/eurheartj/ehac244
40. Mallouppas M, Chung R, Ghosh AK, Macklin A, Yellon DM, Walker JM (2023) Anthracyclines and Biomarkers of Myocardial Injury: The Effect of Remote Ischemic Conditioning. JACC CardioOncol 5:343-355 doi:10.1016/j.jaccao.2023.03.008
41. Maulik A, Davidson SM, Piotrowska I, Walker M, Yellon DM (2018) Ischaemic Preconditioning Protects Cardiomyocytes from Anthracycline-Induced Toxicity via the PI3K Pathway. Cardiovasc Drugs Ther 32:245-253 doi:10.1007/s10557-018-6793-y
42. Moreno-Arciniegas A, Cadiz L, Galan-Arriola C, Clemente-Moragon A, Ibanez B (2024) Cardioprotection strategies for anthracycline cardiotoxicity. Basic Res Cardiol doi:10.1007/s00395-024-01078-6
43. Moreno-Arciniegas A, Garcia A, Kelm M, D'Amore F, da Silva MG, Sanchez-Gonzalez J, Sanchez PL, Lopez-Fernandez T, Cordoba R, Asteggiano R, Camus V, Smink J, Ferreira A, Kersten MJ, Bolanos N, Escalera N, Pacella E, Gomez-Talavera S, Quesada A, Rossello X, Ibanez B, Investigators RT (2024) Rationale and design of RESILIENCE: A prospective randomized clinical trial evaluating remote ischaemic conditioning for the prevention of anthracycline cardiotoxicity. Eur J Heart Fail 26:2213-2222 doi:10.1002/ejhf.3395
44. Omland T, Heck SL, Gulati G (2022) The Role of Cardioprotection in Cancer Therapy Cardiotoxicity: JACC: CardioOncology State-of-the-Art Review. JACC CardioOncol 4:19-37 doi:10.1016/j.jaccao.2022.01.101
45. Rivero-Santana B, Saldana-Garcia J, Caro-Codon J, Zamora P, Moliner P, Martinez Monzonis A, Zatarain E, Alvarez-Ortega C, Gomez-Prieto P, Pernas S, Rodriguez I, Buno Soto A, Cadenas R, Palacios Ozores P, Perez Ramirez S, Merino Salvador M, Valbuena S, Fernandez Gasso L, Juarez V, Severo A, Terol B, de Soto Alvarez T, Rodriguez O, Brion M, Gonzalez-Costello J, Canales Albendea M, Gonzalez-Juanatey JR, Moreno R, Lopez-Sendon J, Lopez-Fernandez T (2024) Anthracycline-induced cardiovascular toxicity: validation of the Heart Failure Association and International Cardio-Oncology Society risk score. Eur Heart J doi:10.1093/eurheartj/ehae496
46. Skyschally A, Kleinbongard P, Lieder H, Gedik N, Stoian L, Amanakis G, Elbers E, Heusch G (2018) Humoral transfer and intramyocardial signal transduction of protection by remote ischemic

perconditioning in pigs, rats, and mice. Am J Physiol Heart Circ Physiol 315:H159-H172 doi:10.1152/ajpheart.00152.2018

47. Thielmann M, Kottenberg E, Kleinbongard P, Wendt D, Gedik N, Pasa S, Price V, Tsagakis K, Neuhauser M, Peters J, Jakob H, Heusch G (2013) Cardioprotective and prognostic effects of remote ischaemic preconditioning in patients undergoing coronary artery bypass surgery: a single-centre randomised, double-blind, controlled trial. Lancet 382:597-604 doi:10.1016/S0140-6736(13)61450-6
48. Totzeck M, Schuler M, Stuschke M, Heusch G, Rassaf T (2019) Cardio-oncology - strategies for management of cancer-therapy related cardiovascular disease. Int J Cardiol 280:163-175 doi:10.1016/j.ijcard.2019.01.038
49. Vinas-Mendieta AE, Gallardo-Grajeda A, Lopez-Fernandez T (2025) Cardio-oncology: chances and challenges. Basic Res Cardiol 120:3-9 doi:10.1007/s00395-024-01080-y
50. Yamamoto Y, Hoshino Y, Ito T, Nariai T, Mohri T, Obana M, Hayata N, Uozumi Y, Maeda M, Fujio Y, Azuma J (2008) Atrogin-1 ubiquitin ligase is upregulated by doxorubicin via p38-MAP kinase in cardiac myocytes. Cardiovasc Res 79:89-96 doi:10.1093/cvr/cvn076
51. Yang AY, Lee JH, Shu L, Zhang C, Su ZY, Lu Y, Huang MT, Ramirez C, Pung D, Huang Y, Verzi M, Hart RP, Kong AN (2014) Genome-wide analysis of DNA methylation in UVB- and DMBA/TPA-induced mouse skin cancer models. Life Sci 113:45-54 doi:10.1016/j.lfs.2014.07.031
52. Yeh ET, Chang HM (2016) Oncocardiology-Past, Present, and Future: A Review. JAMA Cardiol 1:1066-1072 doi:10.1001/jamacardio.2016.2132
53. Yellon DM, Walker JM, Arjun S (2019) Preventing the Cancer Patient of Today From Becoming the Heart Failure Patient of Tomorrow. JACC CardioOncol 1:235-237 doi:10.1016/j.jaccao.2019.11.005

FIGURE LEGENDS

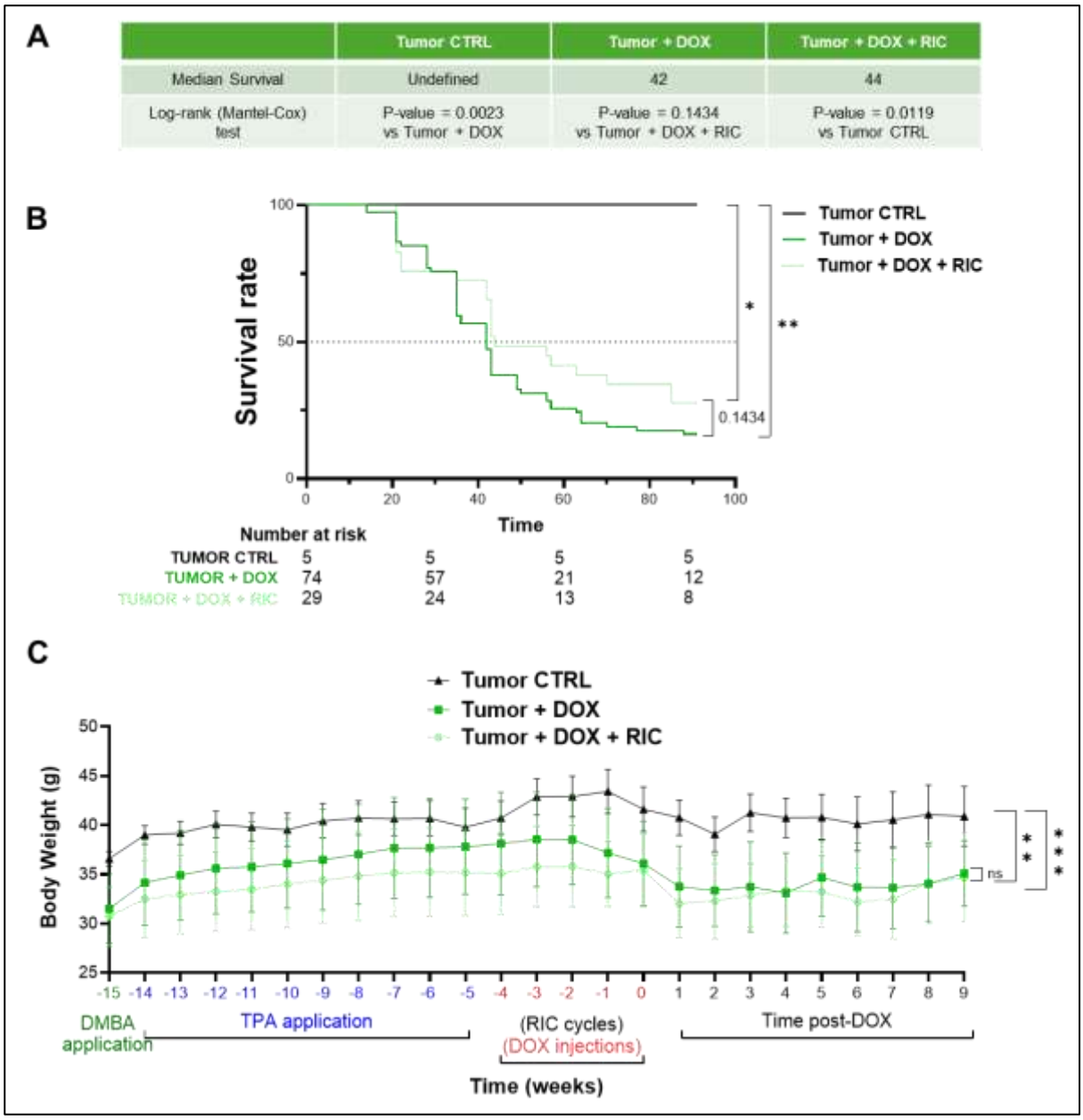
Fig. 1. Study Design.



A, Timeline of the experimental protocol. CD1 mice (4 weeks old) underwent chemical skin carcinogenesis through a single topical application of DMBA (1 g/L, 100 µL), followed by twice-weekly applications of TPA (100 µM, 150 µL) for 10 consecutive weeks. After tumor induction, mice received intraperitoneal doxorubicin (5 mg/kg/week for 5 weeks) with or without concomitant RIC. RIC was performed once weekly and consisted of three cycles of 5-minute hindlimb ischemia followed by 5

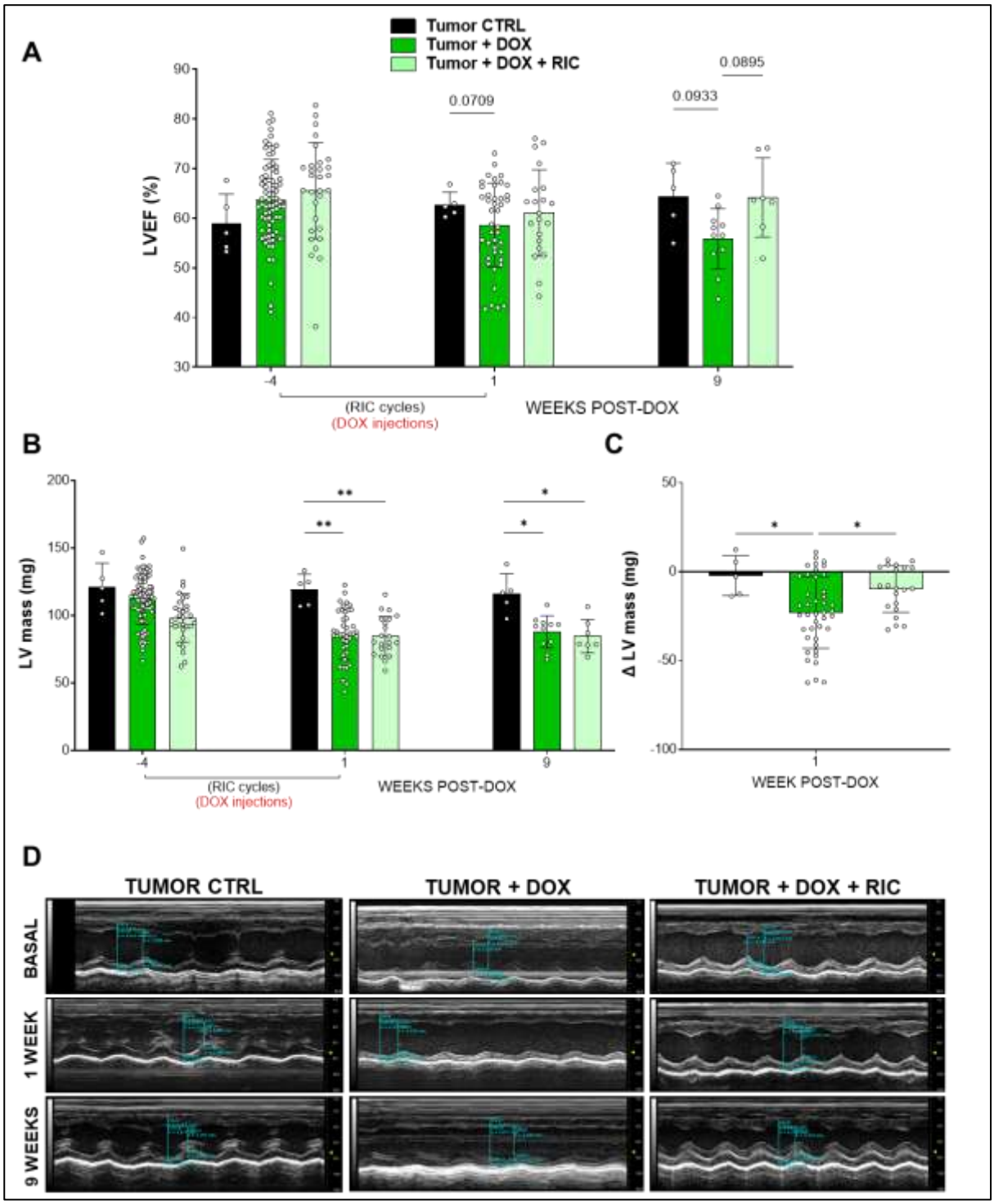
minutes of reperfusion under sevoflurane anesthesia. Echocardiography was conducted at baseline (pre-DOX) and at multiple timepoints throughout treatment and follow-up. **B,** Experimental groups. A total of 110 CD1 mice were included in the study: Tumor CTRL (n=5), Tumor+DOX (n=75), and Tumor+DOX+RIC (n=30). DMBA = 7,12-dimethylbenz[a]anthracene; DOX = doxorubicin; i.p. = intraperitoneal; RIC = remote ischemic conditioning; TPA = 12-O-tetradecanoylphorbol-13-acetate.

Fig. 2. Lifespan of the protocol.



A, Summary of survival data. Median survival and log-rank (Mantel–Cox) test results comparing the three experimental groups. **B,** Kaplan–Meier survival curves (n = 5–74). **C,** Longitudinal evolution of body weight (g) throughout tumor induction, doxorubicin treatment, and follow-up. Data were analyzed using two-way repeated-measures ANOVA (n = 5–74) and are presented as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001. Abbreviations as in Figure 1.

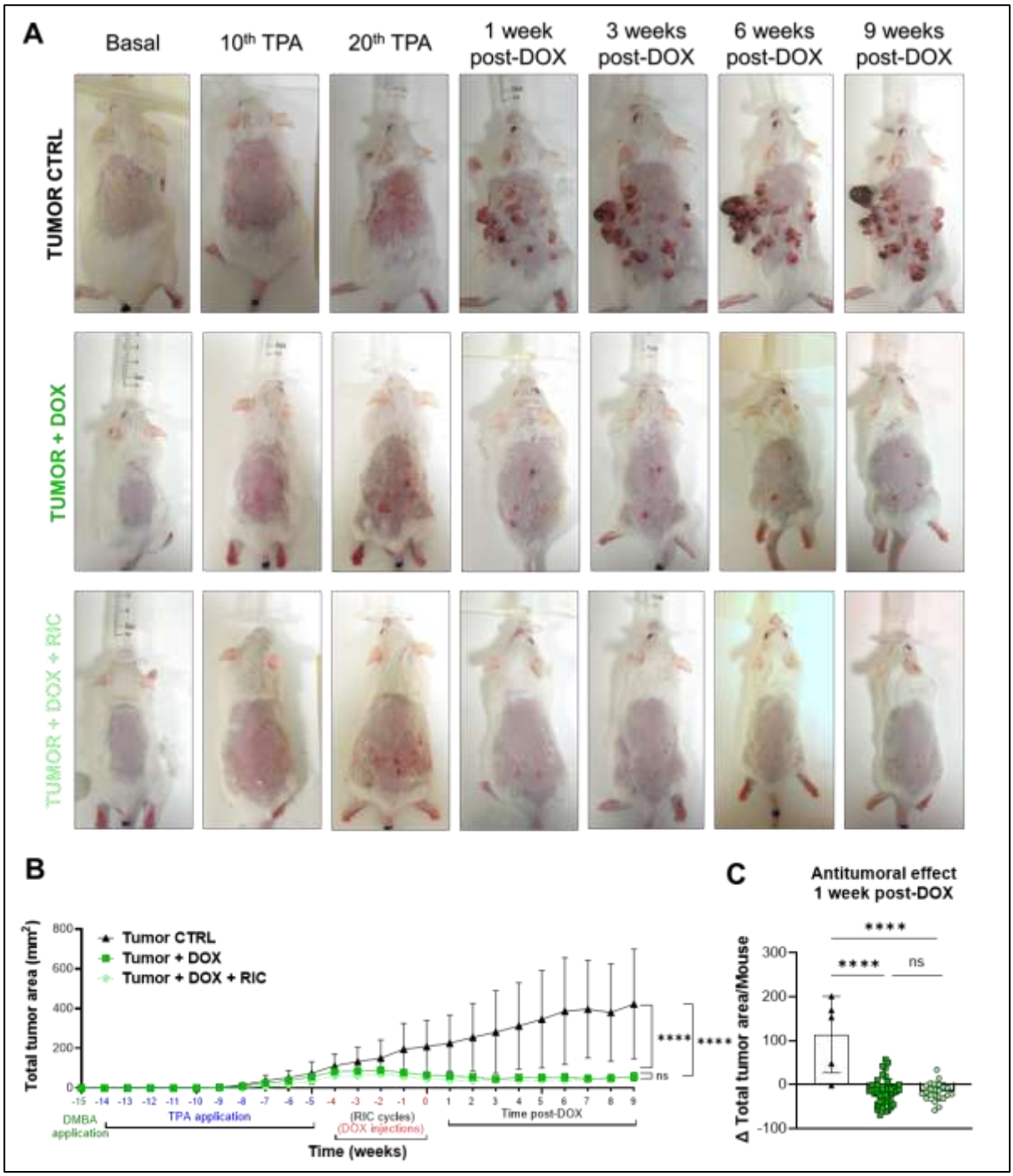
Fig. 3. Trajectories of cardiac function across study duration.



A, Quantification of LVEF (%) by transthoracic echocardiography acquired at baseline (Pre-DOX) and at 1 and 9 weeks post-treatment in the three experimental groups. Data were analyzed using two-way repeated-measures analysis of variance

(ANOVA) followed by Tukey's post hoc test (n = 5–74). **B,** Quantification of LV mass (mg) at the same timepoints and with the same statistical approach as in panel A. **C,** Early change in LV mass (Δ LV mass at 1 week vs baseline) in the three experimental groups. Data were analyzed using one-way repeated-measures analysis of variance (ANOVA) followed by Tukey's post hoc test (n = 5–42). **D,** Representative M-mode echocardiographic images over time. Data are presented as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001. LV = left ventricle; LVEF = left ventricular ejection fraction; other abbreviations as in Figure 1.

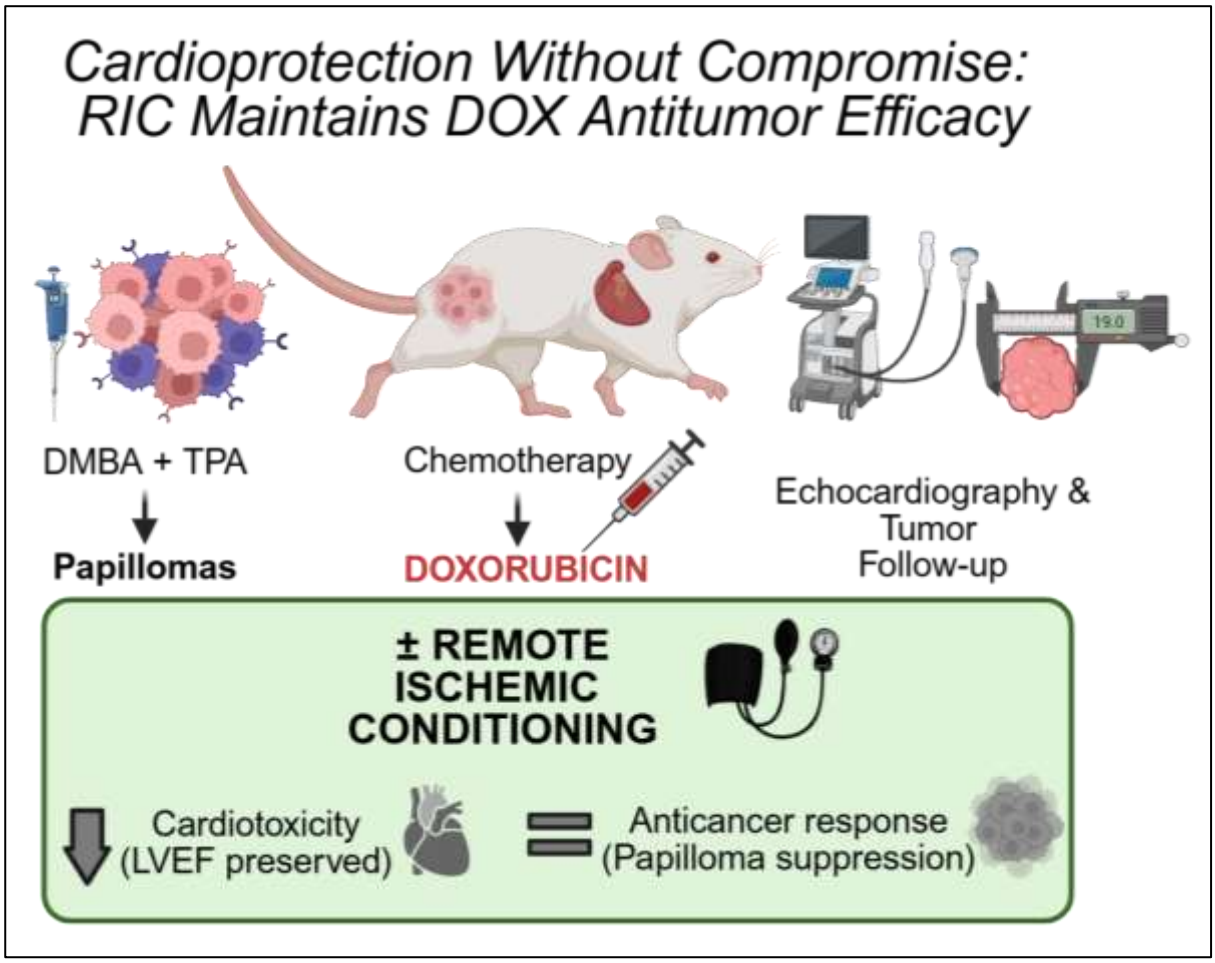
Fig. 4. Tumor growth.



A, Representative dorsal skin images at baseline, after 10 and 20 topical applications of TPA, and at 1, 3, 6, and 9 weeks following initiation of doxorubicin treatment. **B,** Quantification of total tumor area per mouse over time. Data were analyzed using two-way repeated-measures ANOVA followed by Šídák's post hoc test (n = 5–75) and are presented as mean ± SEM. **C,**

Acute antitumoral effect of treatment measured as change in total tumor area one week after the first doxorubicin injection, compared to pre-treatment baseline. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test (n = 5–43) and are presented as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Abbreviations as in Figure 1.

Summary Figure. Cardioprotection Without Compromise: *RIC maintains DOX Antitumor Efficacy*.



Schematic representation of the experimental design and main findings. Skin papillomas were induced in CD1 mice through topical application of DMBA followed by repeated TPA treatments. After tumor development, animals received five weekly intraperitoneal injections of doxorubicin (DOX) with or without concomitant remote ischemic conditioning (RIC). RIC consisted of three cycles of 5-min hindlimb ischemia followed by 5-min reperfusion, performed weekly under anesthesia. Cardiac and oncological outcomes were monitored by serial echocardiography and tumor imaging. RIC reduced DOX-induced cardiotoxicity, preserving LVEF and partially attenuating LV atrophy, while maintaining the antitumor efficacy of chemotherapy as reflected by equivalent papilloma suppression in both DOX and DOX + RIC groups. DMBA = 7,12-dimethylbenz[a]anthracene; LVEF = left ventricular ejection fraction; RIC = remote ischemic conditioning; TPA = 12-O-tetradecanoylphorbol-13-acetate.

Table 1. Echocardiographic Parameters.

Comparison of cardiac function and structural parameters measured by transthoracic echocardiography (M-mode) in Tumor CTRL, Tumor+DOX, and Tumor+DOX+RIC mice at baseline (post-TUMOR, pre-DOX), 1 week and 9 weeks after completion of DOX treatment (n=5-74). Data are presented as mean ± SD. Parameters include ejection fraction (EF), fractional shortening (FS), left ventricular (LV) mass, and LV volumes during diastole (LV Vol;d) and systole (LV Vol;s). DOX = doxorubicin; EF = ejection fraction; FS = fractional shortening; LV = left ventricle; RIC = remote ischemic conditioning; Vol = volume; d = diastole; s = systole.

TUMOR CTRL				
Parameter	*Unit*	*Basal (Post-TUMOR, Pre-DOX) (n=5)*	*1 week (n=5)*	*9 weeks (n=5)*
EF	**%**	**58.87 ± 6.00**	**62.73 ± 2.53**	**64.46 ± 6.64**
FS	%	31.15 ± 4.30	33.54 ± 1.88	34.93 ± 4.64
LV mass	mg	151.81 ± 21.64	149.14 ± 14.33	145.29 ± 18.50
LV mass (corrected)	**mg**	**121.45 ± 17.31**	**119.31 ± 11.47**	**116.23 ± 14.80**
LV Vol;d	uL	90.32 ± 9.35	74.66 ± 9.83	74.07 ± 19.62
LV Vol;s	uL	36.91 ± 4.95	27.82 ± 4.16	27.05 ± 11.21
TUMOR + DOX				
Parameter	*Unit*	*Basal (Post-TUMOR, Pre-DOX) (n=74)*	*1 week (n=42)*	*9 weeks (n=11)*
EF	**%**	**63.75 ± 8.13**	**58.64 ± 8.46**	**55.82 ± 6.10**
FS	%	34.64 ± 5.87	30.80 ± 5.48	28.79 ± 3.82
LV mass	mg	138.51 ± 27.55	107.65 ± 23.07	109.88 ± 14.98
LV mass (corrected)	**mg**	**112.21 ± 18.82**	**86.12 ± 18.46**	**87.88 ± 12.00**
LV Vol;d	uL	80.86 ± 17.28	69.53 ± 20.47	69.69 ± 15.56
LV Vol;s	uL	29.92 ± 10.70	29.83 ± 13.26	31.35 ± 10.47
TUMOR + DOX + RIC				
Parameter	*Unit*	*Basal (Post-TUMOR, Pre-DOX) (n=30)*	*1 week (n=21)*	*9 weeks (n=7)*
EF	**%**	**65.53 ± 9.67**	**61.10 ± 8.70**	**64.18 ± 7.97**
FS	%	36.08 ± 7.13	32.61 ± 6.16	34.64 ± 5.84
LV mass	mg	122.94 ± 22.56	106.00 ± 18.35	105.88 ± 15.49
LV mass (corrected)	**mg**	**98.35 ± 18.05**	**84.80 ± 14.68**	**84.70 ± 12.39**
LV Vol;d	uL	73.70 ± 16.55	70.34 ± 18.47	62.30 ± 17.20
LV Vol;s	uL	26.22 ± 11.89	27.89 ± 10.79	22.71 ± 9.08