Mechanism-Based Engineering against Anthracycline Cardiotoxicity

Running title: Force et al.; Bivalent NRG-1 in cardioprotection

Thomas Force, MD1; Yibin Wang, PhD2

1Dept of Medicine and Center for Translational Medicine, Temple University, Philadelphia, PA;
2Depts of Anesthesiology, Physiology and Medicine, David Geffen School of Medicine,
University of California, Los Angeles, Los Angeles, CA

Address for Correspondence:
Yibin Wang, PhD
University of California, Los Angeles
BH 569, CHS
650 Charles E. Young Drive
Los Angeles, CA 90095
Tel: 310-206-5197
Fax: 310-206-5197
E-mail: yibinwang@mednet.ucla.edu

Journal Subject Codes: Basic science research:[131] Apoptosis, Heart failure:[11] Other heart failure

Key words: Doxorubicine, Neuregulin-1beta, topoisomerase 2, bivalent ligand, cardioprotection, cancer and stroke, Editorial
Since their first discovery nearly 50 years ago, anthracyclines, including doxorubicin (Adriamycin), daunorubicin (Cerubidine), epirubicin (Ellence) and idarubicin (Idamycin PFS) have been successfully developed as potent anti-cancer therapeutics with significant efficacy in lymphomas and many solid tumors. Particularly in patients with breast cancer, they are the primary choices of therapy. However, cardiotoxicity has been a central limiting complication in treating patients since the agents acutely produce arrhythmias, LV dysfunction, and pericarditis, and chronically lead to LV dysfunction and heart failure. The toxicity is clearly dose-related with sharp rises in LV dysfunction with cumulative doses above 400-450 mg/m² for doxorubicin. When cardiac imaging was employed, the incidence of HF was 5%, 26%, and 48% in patients receiving 400, 550, and 700 mg/m² of doxorubicin. As a result, most oncologists typically limit the dose to 450-500 mg/m². Children are especially vulnerable with rates of significant LV dysfunction of 5% at 15 yrs of follow-up, increasing to 10% for cumulative doses of ≥550 mg/m². Heart failure may present many years after treatment. Mediastinal irradiation is an additional risk factor that may also be particularly problematic in children. To date, our only proven protective measure is adherence to “stopping guidelines” for total dose. Unfortunately, this typically limits the total dose an individual patient could receive, and for particularly problematic cancers, oncologists would like to use higher doses.

This issue may be particularly problematic in patients with breast cancer who are positive for human epidermal growth factor receptor 2 (Her2). This receptor is amplified in ~30% of breast cancers, leading to activation of signaling pathways that promote proliferation of the tumor cells and block tumor cell death. Initial strategies for treatment included concurrent anthracycline and the anti-Her2 monoclonal antibody, trastuzumab (Herceptin). However this led to a high incidence of cardiotoxicity. Trastuzumab is now administered after completion of
anthracycline dosing, and that has reduced the incidence of heart failure\textsuperscript{7,8}. Despite all of the above machinations to reduce cardiotoxicity of anthracyclines and trastuzumab, this still remains a thorn in the side of the oncologist and cardiologist. Clearly, strategies to limit cardiotoxicity while maintaining anti-cancer efficacy are desperately needed.

Earlier studies have uncovered a number of mechanisms involved in anthracycline-induced cardiac injury\textsuperscript{9,10}. The most extensively characterized mechanism is anthracycline-induced reactive oxygen species (ROS) and subsequent oxidative stress-induced DNA damage, mitochondrial dysfunction, sarcomere damage and loss of pro-survival signaling. Unfortunately, numerous approaches focusing on the use of antioxidants were generally ineffective, due at least in part to the fact that ROS-induced cytotoxicity is a shared mechanism for both cardiotoxicity and tumoricidal activity. Consequently, our history of identifying novel strategies to prevent cardiotoxicity while preserving anti-cancer efficacy has a very checkered past with few (or no) true successes\textsuperscript{11}.

However, recent findings from the Yeh laboratory\textsuperscript{12} and the Lee laboratory reported in this issue of \textit{Circulation}\textsuperscript{13} offered two novel approaches to treat or prevent doxorubicin-induced cardiomyopathy (\textbf{Figure 1}). Although starting from very different vantage points, both laboratories exploited the subtle but important molecular differences between cardiomyocytes and cancer cells. In both cases, these differences were used as the mechanistic basis to develop strategies to protect cardiomyocytes against Dox treatment without affecting its anti-cancer efficacy. It is indeed remarkable and exciting that novel, and possibly game-changing approaches to an age-old conundrum (anthracycline induced cardiotoxicity) were identified within a few months of one another.

In the report by Zhang \textit{et al}, a critical role of topoisomerase-2β (Top2β) in anthracycline...
cardiotoxicity was discovered based on studies in both cultured cells and intact animals. They demonstrated that Dox-mediated DNA damage and subsequent mitochondrial defects were Top2β dependent processes in cardiomyocytes\textsuperscript{12}. Dox-induced genomic DNA strand breaks, mitochondrial loss, cardiomyocyte death, and eventual LV dysfunction were markedly reduced in Top2β KO mice. Since Top2β is selectively expressed in heart, but Top2α is absent, a β isoform-specific inhibition of Top2 may offer a novel strategy to prevent Dox induced cardiotoxicity without affecting its anti-cancer activities if Top2α remains functional in cancer cells. This finding also breathes interesting new life into an age-old debate over whether, and/or how, dexrazoxane, an iron-chelator and topoisomerase inhibitor, is cardioprotective against Dox treatment\textsuperscript{14,15}. Indeed, after so many years of clinical observation, revealing the functional role of topoisomerase 2β in Dox-mediated cardiac injury might offer a bona fide mechanism for the beneficial effect of dexrazoxane. Largely because of that, another trial in the clinic with dexrazoxane or similar compounds is likely.

The report in this issue of Circulation from the Lee lab offered yet, another novel approach to achieve biased protection in cardiomyocytes against Dox treatment\textsuperscript{13}. Neuregulin-1β (NRG1B) functions through its receptors, ErbB2 and ErbB4, to exert potent cardioprotection\textsuperscript{16} against Dox-induced injury\textsuperscript{17}. However, it can also induce oncogenic activity through receptor-mediated signaling in cancer cells. This poses an especially critical dilemma for breast cancer patients, even with staged Dox/trastuzumab therapy. Interestingly, ErbB4 is enriched in cardiomyocytes and can transduce protective signaling through homodimer interaction, while cancer cells express ErbB3 which requires ErbB3/ErbB2 heterodimer interaction to transduce downstream oncogenic effect. Lee and his colleagues exploited this property using a modified NGR1B ligand\textsuperscript{18}. By tethering two molecules with a linker, this
bivalent NRG1B ligand (NN) has a strong preference for homodimer signaling over heterodimer signaling in the target cells\(^{18}\). In both in vitro and in vivo, the newly engineered NN showed a potent cardioprotective effect against Dox treatment (where ErbB4 homodimers signal downstream to pro-survival pathways) and also demonstrated significantly reduced pro-growth signaling and pro-neoplastic potential in cancer cells (where ErbB4 homodimers fail to signal to downstream pathways) \(^{13}\).

Despite these exciting advancements, clinical translation of these newly established cardioprotective strategies need to be further validated. Both studies showed potent protective effects to attenuate Dox-induced cardiomyopathy in mice, but did not provide \textit{in vivo} evidence of preserving anti-cancer potency in an animal model. Since Dox-induced cardiomyopathy can develop many years after treatment, longer term observation will also be critical. Nevertheless, these two reports represent significant advances in our current therapeutic approaches to anthracycline-induced cardiomyopathy. They also highlight the importance of better understanding of the disease processes and the therapeutic agents at a mechanistic and fundamental level in order to achieve rational design of therapies. These two successes demonstrate again that mechanism based engineering (genetic or protein) holds great promise to tackle complex and challenging diseases in addition to cardiotoxicity of cancer therapies.

**Acknowledgements:** The authors thank Ms. Wendy Buck for her kind assistance in manuscript preparation.

**Funding Sources:** This work was supported in part by National Institutes of Health grants HL070079, HL103205, HL108186, HL098954 (YW) and HL61688, HL114124, HL091799 (TF).

**Conflict of Interest Disclosures:** None.
References:


**Figure Legend:**

**Figure 1.** Novel strategies in cardioprotection against anthracycline induced cardiotoxicity.

Mechanism based engineering of biased NRG ligand and topoisomerase 2β inhibition prevents Dox induced cardiomyocyte death.
Mechanism-Based Engineering against Anthracycline Cardiotoxicity
Thomas Force and Yibin Wang

Circulation. published online June 11, 2013;
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/early/2013/06/11/CIRCULATIONAHA.113.003688

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/