The human atrial myocardial growth factor receptor (HER2) signaling pathway is amplified and overexpressed in 20 to 30% of breast cancers and is associated with poor prognosis. While the 3 current HER2-targeted therapies (lapatinib and trastuzumab) show clinical benefit, the development of resistance and cardiotoxicity has limited their use. These concerns have led to the development of pan-HER inhibitors, which may improve clinical efficacy. However, this is known to cause the cardiotoxicity of these therapies. This study sought to examine the safety profile of the pan-HER inhibitors afatinib and neratinib, alone or in combination, in combination with the anthracycline doxorubicin on hiPS cardiac cell-derived cardiomyocytes (hiPS-CM). Treatment with atelisocib and trastuzumab alone caused cardiac damage at concentrations well above IC50 of their respective HER2 concentrations. Doxorubicin potentiated the effect of lower concentrations (5-10% IC50) of both pan-HER inhibitors, exacerbating the effects on both functional and structural cardiotoxicity, in hiPS-CM.

Results

**Combination treatment with low dose of either afatinib or neratinib plus doxorubicin significantly decreases cell viability of hiPS-CM**

Human iPSC-CM were treated for 1 hr with a pan-HER inhibitor followed by treatment with 1.0 µM doxorubicin for an additional 24 hr (total time = 25 hr). The combination of the pan-HER inhibitors was set at 50% following the addition of doxorubicin. Doxorubicin alone led to a 40% decrease in cell viability. Both afatinib and neratinib potentiated the effect of doxorubicin at both tested dose concentrations, as compared to the control. Although neratinib did not significantly decrease cell viability of doxorubicin of either tested dose combination alone (≤40% decrease in cell viability), when combined with doxorubicin, a synergistic effect was observed, as compared to the control. No significant effect on cell viability was observed when pan-HER inhibitors were combined with an anthracycline.

**Afatinib and neratinib lead to alterations in cardiac cell beating as detected using the xCELLigence real-time monitoring platform.**

**Afatinib and neratinib inhibit iPSC-CM viability at high concentrations**

Analysis of the effects of the drugs on cardiac cell beat activity. All values were double-normalized to an internal baseline time point.

**Doxorubicin potentiates the effect of afatinib on cardiac cell beating**

Doxorubicin potentiates the effect of lower concentrations (5-10% IC50) of both pan-HER inhibitors, exacerbating the effects on both functional and structural cardiotoxicity, in hiPS-CM.

**Conclusion**

- Afatinib and neratinib are relatively cardiac safe at doses near their Cmax values (152 nM and 152 nM, respectively). However, cardiototoxicity is evident upon treatment with higher doses (≥200 nM) of each pan-HER inhibitor, suggesting caution should be used in the adjusted setting or with long-term use.
- Combination treatment with lower doses of pan-HER inhibitors followed by exposure to the anthracycline doxorubicin leads to increased cardiotoxicity as evidenced by significant alterations in cardiac cell beating activity, increased lipid accumulation suggestive of mitochondrial damage, and decreased cell viability.
- Targeted oncology therapies such as afatinib and neratinib may induce the survival pathway in cardiomyocytes, leading to greater susceptibility to doxorubicin-related damage.
- Since many targeted therapies are given in combination with other oncology treatments, comprehensive safety screening for combination therapies is warranted to illuminate any potential synergies on cardiotoxicity.
- This study shows the ability of a multi-parameter in vitro screening using human induced cardiomyocytes (hiPS-CM) to detect potential cardiac risk for both single drug and novel combination therapies.
- Future studies will investigate the cardiotoxic effects of pan-HER inhibitors with standard-of-care chemotherapeutics (e.g., paclitaxel, carboplatin) to further refine in vitro cardiotoxic risk assessment.