Introduction The ERK1/2 cascade, a key pathway involved in cardiac remodelling, is regulated by RAF kinases. Small molecule inhibitors of RAF have been developed due to activating oncogenic mutations, however paradoxical activity has been seen in early generations of inhibitors. Therefore, ‘paradox breaker’ inhibitors (e.g. PLX8394) have been developed and are undergoing clinical trials. Here, we investigated the effects of PLX8394 on vascular ERK1/2 signalling in vitro and on hypertensive cardiac remodelling in vivo.

Methods Murine endothelial cells (ECs) or human cardiac fibroblasts (HCFs) were incubated with PLX8394 and effects on RAF-ERK1/2 pathway activity determined by western blotting, with effects on cell migration and proliferation assessed via wound healing and BrdU assays. For in vivo characterisation, PLX8394 (5mg/kg/d) was infused with/without angiotensin-II (AngII; 0.8mg/kg/d) for 7 days by osmotic minipumps in male wildtype C57Bl/6J mice (n=8–11/group). Cardiac function/dimensions were assessed using echocardiography; effects on cardiac morphology were assessed by histological staining. mRNA expression was assessed by qPCR. Statistical tests used 1-way ANOVA with Holm-Sidak’s post-test.

Results PLX8394 (5 min; 1μM) activated ERK1/2 (n=3; p=0.018) pathway via CRAF (n=3; p=0.047) in ECs with no change seen in BRAF activity. This was accompanied by increased BrdU incorporation (n=6; p=0.0002; p=0.0009) but significantly inhibited migration (n=6; p<0.0001; p<0.0001) both at baseline and with AngII (100nM), respectively. In HCFs however, PLX8394 had no effect on baseline or AngII migration (n=4; p=0.99; p=0.98) or BrdU incorporation (n=6; p=0.95; p=0.65). In vivo, PLX8394 did not alter the AngII-induced cardiac hypertrophy with maintained wall thickness to internal diameter ratio (p=0.45). While PLX8394 was able to significantly reduce cardiomyocyte cross sectional area (p=0.0068), no changes were seen in Myh7, Nppa or Nppb mRNAs. Moreover, PLX8394 did not significantly alter the perivascular (p=0.69) or interstitial (p=0.052) fibrotic area with no changes in mRNA expression of collagens1-4.

Conclusion PLX8394, despite development as a cancer cell ‘paradox breaker’, activates ERK1/2 signalling in ECs, but not HCFs. In vivo, PLX8394 had minimal effect on hypertensive cardiac fibrotic remodelling despite reducing myocyte hypertrophy, likely reflecting a cell-type dependent response. Thus, paradox-breaker RAF inhibitors, currently in clinical trials for RAF-mutant cancers, may have limited viability as hypertension therapies.