Specificity of ENC2015 was established with co-infusion of the Factor XHa inhibitor, iodoacetamide. 18F-ENC2015 biodistribution, kinetics, radiometabolism and ability to bind to an acutely thrombosed artery in vivo were characterised in rats.

**Results** Both Cy5 and fluorine-18 labelled ENC2015 rapidly and specifically bound to both low and high shear thrombi. Both thrombus fluorescence and PET radioactivity was inhibited by a factor XHa inhibitor. There was no metabolism of 18F-ENC2015 for over 8 hours when incubated ex vivo in whole human blood. In vivo, approximately 42.0% of parent radiotracer remained in the blood 60 min post-administration. Biodistribution studies demonstrated low uptake and rapid clearance from tissues with elimination via the urinary system. In an in vivo rat model of arterial thrombosis, 18F-ENC2015 uptake was markedly increased in the thrombosed carotid artery compared to the contralateral patent artery (mean standard uptake value ratio of 2.40 versus 0.74, p<0.0001).

**Conclusion** ENC2015 rapidly and selectively binds to acute thrombus in both an ex vivo human translational model and an in vivo rodent model of arterial thrombosis. This probe holds promise for the non-invasive identification of thrombus formation in cardiovascular disease.

Figure 1. Biodistribution and thrombus binding of 18F-ENC2015 in rats. Panel A shows images of the time averaged uptake within the thrombus and skin incision compared with the contralateral vessel. Panel B is a decay corrected time activity curve showing 18F-ENC2015 kinetics (mean SUV, n=3) within our in vivo thrombosis model. Note the rapid blood pool clearance of 18F-ENC2015 with early uptake in the skin incision which tails off to equilibrate with the arterial thrombus between 60 and 90 mins. Panel C demonstrates the mean change in SUV ratio of the thrombosed vessel compared with contralateral vessel and LV blood pool over time (n=3). Thrombus vs contralateral vessel SUV from 35 to 180 minutes p value <0.0001 (paired t-test).

**Conflict of Interest** None