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## **Biosketch**

Dr Dan Antoine is currently a Wellcome Trust funded research fellow at the MRC Centre for Drug Safety Science (CDSS) and lecturer in Pharmacology at the University of Liverpool, UK. Dan completed his PhD in 2009 in Pharmacology and prior to this he worked in research posts within Molecular Toxicology at AstraZeneca. He undertook postdoctoral training at the CDSS as well as Royal Society International Travelling Fellowships at the Harvard Medical School, USA. Dan is currently the coordinator of the safety biomarker research group at the CDSS, Liverpool, UK. His research is mainly focused on the understanding of fundamental mechanisms related to drug-induced liver injury (DILI) and the development of novel DILI biomarkers and therapeutic interventions. He sits on the British Toxicology Society's (BTS) Education, Training and Early Career Toxicologists Sub-Committee and is the co-chair of the British Pharmacological Society (BPS) Toxicology Affinity Group. In 2014, Dr Antoine was elected to serve as a Councillor for the International Union of Basic and Clinical Pharmacology (IUPHAR) Drug Metabolism and Drug Transport Section Executive Board and from 2016 will serve on the Hepatotoxicity SIG steering committee for the American Association for the Study of Liver Diseases (AASLD). Dr Antoine has received a number of early career new investigator awards for his research. In 2013, he received the BTS Early Career Investigator Award and in 2015 was awarded the European New Investigator Award from the International Society for the Study of Xenobiotics (ISSX), the 2015 Bill Bowman Travelling Lectureship from BPS and the 2015 Young Investigator Award in Translational Pharmacology jointly from the Federation of European Pharmacological Societies (EPHAR) & the European Association for Clinical Pharmacology and Therapeutics (EACPT). In 2016 he was awarded the Outstanding Young Investigator Award jointly from BPS & the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists (ASCEPT). Dan is an editorial board member for the journals *Pharmacology Research & Perspectives* and *Biomarkers*. His academic research is currently funded by grants awarded from the European Commission, Medical Research Council, Wellcome Trust, The Royal Society and the pharmaceutical industry.

## **Abstract: Targeting HMGB1 with antibodies and inhibitory peptides**

The danger signal, High Mobility Group Box-1 (HMGB1), is a chromatin binding protein that sits at the intersection between sterile and infectious immunity. We have previously shown that HMGB1 signaling is critical to drive the pathogenic process of experimental drug-induced liver injury (DILI) using novel hepatocyte conditional knock out mice [1]. HMGB1 is passively released by necrotic cells and acts as a DAMP (Damage Associated Molecular Pattern) that connects cell death with the activation of immune responses. Active secretion of HMGB1 also occurs via acetylation of key nuclear localisation signals. The function of HMGB1 as an inflammatory mediator is highly regulated by post-translational redox modifications. We have also demonstrated that the acetyl isoform of HMGB1 is a prognostic biomarker of clinical acetaminophen overdose [2] and deleterious outcome in human idiosyncratic DILI. Furthermore redox-dependent and functionally relevant HMGB1 isoforms also show characteristic signatures associated with outcome and can distinguish between benign or serious liver injury in man and in vivo. Given that these data point to HMGB1 being a master regulator of clinical and experimental DILI, we reasoned that HMGB1 would represent a novel and attractive therapeutic target. We developed partly humanized monoclonal antibodies targeted towards HMGB1 and inhibitory peptides based on in silico predictions of HMGB1 key binding residues for

interactions with target receptors (TLR4-MD2) [3]. Data to be presented here show that our lead monoclonal antibody (h2G7) was dose dependently efficacious in preclinical acetaminophen hepatotoxicity models as shown by reduced ALT activity, improved survival and by blocking inflammatory cytokine upregulation and hepatic inflammatory cell infiltrates. We have also shown that the mechanism of protection was due to analyte neutralisation through the use of effector function deficient variants of h2G7 required for complement activation and antibody dependent cell cytotoxicity. In parallel we have also demonstrated that the inhibitory peptides were also protective in vivo. Here we also show these therapeutics were also efficacious at later time points when the acetaminophen antidote, acetylcysteine, was ineffective. These data show for the first time the generation of a partly humanized HMGB1-neutralizing antibody and inhibitory peptides with validated therapeutic efficacy. It represents important progress towards clinical implementation of HMGB1-specific therapy as a means to treat paracetamol overdose and possibly other human inflammation-dependent DILI events.

These studies form part of a collaboration between the MRC Centre for Drug Safety Science (UK), the Institute for Drug Safety Science (USA), the Feinstein Institute (USA) and the Karolinska Institute (Sweden).

[1] P Huebener et al., 2015 J Clin Invest 125:539-50. [2] DJ Antoine et al., 2012 J Hepatology. 56:1070-9. [3] H Yang et al., 2015 J Exp Med 212:5-14