Histological Comparisons of Clinical and Non-clinical of Drug-induced Vascular Injury to Support Qualification of Translational Biomarkers

S. Guionaud, P. Cacoub, M. Lawton, K. Bendjama, H. Firat, and the other members of SafeT "Workpackage 4" (WP4*)

ABSTRACT

Introduction: Drug-induced vascular injury (DIVI) is a common histopathologic observation in animals during safety assessment studies that can stop development if safety margins are insufficient. The **SAFE-T consortium**, formed by representatives of commercial companies and academia, seeks to develop translational safety markers for DIVI via a lesion-based approach of back-translation from man to preclinical species.

Methods: Based on the hypothesis that similar histopathologic lesions in the different compartments of blood vessels are likely to present overlapping biomarker signatures, the SAFE-T group has initiated clinical qualification studies sourcing blood samples from patient groups that present pathologic features similar to the ones observed in preclinical DIVI. Samples are sourced from patients suffering from: Takayasu disease, Behçet's disease, mixed cryoglobulinemia, stent or balloon angioplasty injury, Radiation injury, cutaneous leukoclastic vasculitis, as well as healthy volunteers. Compared features include the vessel type involved, degeneration/ necrosis, vacuolation and proliferation of the endothelium and smooth muscle cells, as well as intensity, composition and location of inflammatory infiltrates.

A detailed comparison of characteristic histologic changes in relevant human diseases and treated rats as well as preliminary biomarker results will be shown.

Results/ Discussion: The comparable aspects of histopathology between the conditions included in the SAFE-T strategy and non-clinical DIVI open the way for a novel biomarker strategy of back-translation.

PROBLEM STATEMENT AND REMIT

Drug-induced vascular (arterial and venular) injury [DIVI] is a relatively common hazard identified during nonclinical toxicity testing which presents a safety assessment dilemma to investigators wishing to assess the safety of new medicines for humans. It is made worse by the gaps in our knowledge concerning pathogenesis and the absence of validated nonclinical or clinical biomarkers (Kerns *et al.*, 2005).

The European Innovative Medicines Initiative in its SAFE-T (Biomarker) call has initiated a new search for translational (predictive and diagnostic) biomarkers with a clear emphasis on **human samples**. Therefore the SAFE-T investigations are commencing with the investigation of human samples first and then back translating to preclinical species. As the mechanism behind preclinical DIVI are little understood, and are thought to be essentially different from vascular injury in humans, **histopathologic changes were identified as the connecting link** between the conditions and a logical indicator of biomarker classes that are worth investigating. Biomarkers are connected to 3 areas of histopathologic change: endothelial reaction, smooth muscle damage and inflammation.

We hypothesize that similar histopathology between preclinical DIVI and vascular injury/disease in humans will lead to overlapping biomarker signatures

Preclinical DIVI versus Drug-induced Vasculitis in Humans

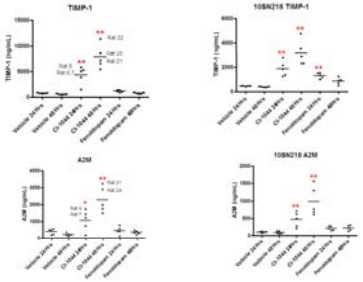
	Preclinical DIVI	Clinical Drug-induced Vasculitis
Symptoms	• None apparent	• Often indistinguishable from idiopathic vasculitides • Arthralgia, myalgia, fever, and arthritis • Sometimes fatal
Organs and vessels affected	• Typically large, medium and small arteries • Frequently mesenteric arteries (rats) and coronary arteries (dog)	• Usually affects small and medium-sized vessels • Generally affects the skin, though sometimes kidney, lung, and other organs
Pathology	• No evidence of anti-neutrophil cytoplasmic antibodies (ANCA)	• Often ANCA negative and sometimes antinuclear antibody (ANA) positive
Time of onset	• From 24 hours to few days after exposure.	• From 3-4 weeks to several months after exposure
Mechanism(s)	• Biomechanical injury induced by blood flow • Direct toxicity to the vascular endothelium • Immune mediated	• Immune mediated (Type I and Type III hypersensitivity)
Drugs	Often vasoactive but many others as well	Examples from nearly every phamaceutical class of drugs (includes antibiotics, antithyroid, antirheumatics, antivirals, etc.)

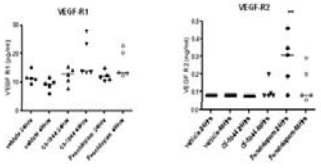
* PSTC Candidate Biomarker

** Selection based on clinical data

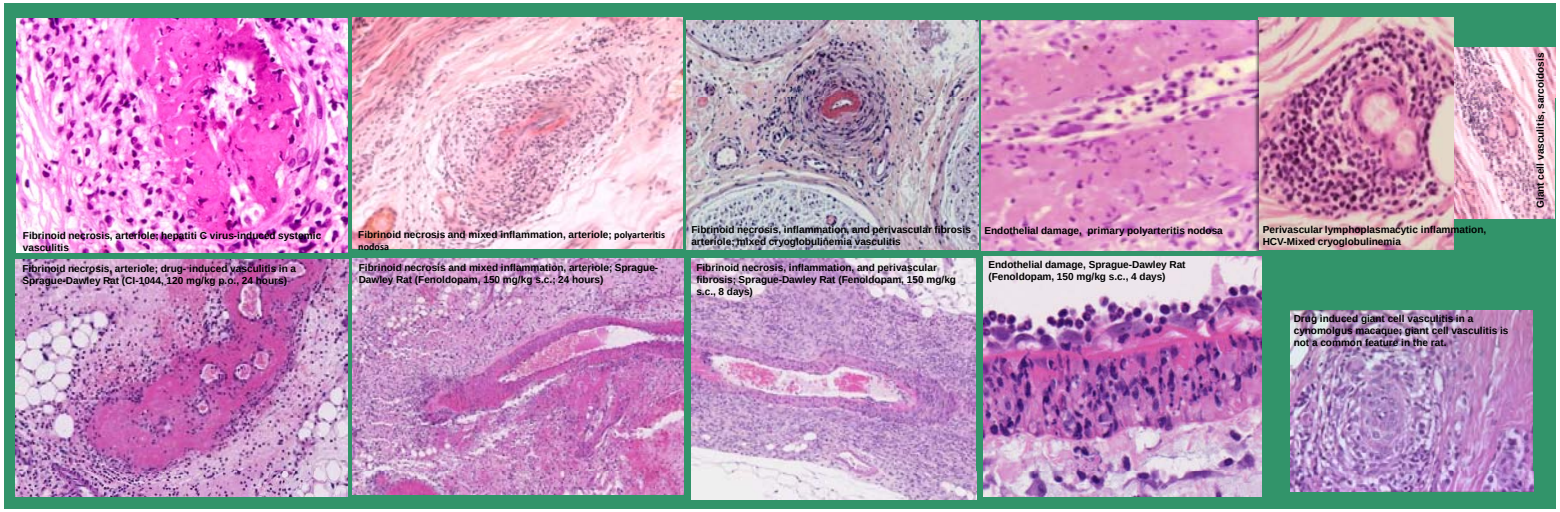
REVERSE QUALIFICATION STUDIES

All candidate biomarkers will also be tested in preclinical (rat) models of DIVI, and where possible in collaboration with the PSTC. The graphs below are for two biomarkers (TIMP-1, alpha 2 macroglobulin or A2M) that were measured in serum from rats were treated with CI-1044 (a PDE4 inhibitor) and fenoldopam (in two separate studies).



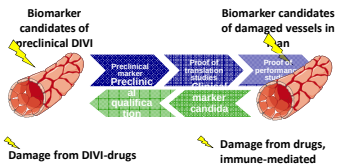


CUSABIO Biotech Co LTD kit for Rat VEGF-R1 and R2

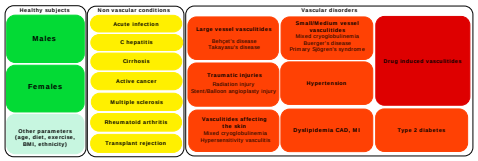


Endothelial Microparticle data from Pfizer to be included here

PARALLEL REVERSE/FORWARD TRANSLATION



GLOBAL OVERVIEW OF POPULATIONS OF INTEREST



In addition to vascular disorders selected on the basis of their histopathologic manifestations, various healthy or non-vascular disease populations have been selected based on the known co-variates affecting the selected biomarker candidates.

CANDIDATE BIOMARKERS

Inflammatory markers	Endothelial markers	Smooth muscle markers
Chemokine ligand 19**	Angiotensin I converting enzyme**	H1-Calponin*
Chemokine ligand 2 (MCP-1)*	Caveolin 1*	H-Caldesmon*
Chemokine ligand 3**	Endothelial cell-specific molecule 1**	SM22/Transgelin*
Chemokine ligand 10**	Endothelial microparticles*	Smooth muscle alpha actin*
Chemokine ligand 11**	Endothelin 1*	Smoothelin*
Chemokine ligand 12**	E-Selectin*	
Chemokine ligand 9**	Intracellular cell adhesion molecule 1*	
C-Reactive Protein**	P-Selectin**	
Interleukin 6*	Thrombomodulin**	
Interleukin 8*	Thrombospondin 1*	
Lipocalin 2*	Vascular cell adhesion molecule 1**	
Serum nitrite*	Vascular endothelium growth factor*	
Tissue inhibitor of metalloproteinase 1*	Von Willebrand factor**	
Transforming growth factor b**	Von Willebrand factor propeptide*	
Tumor necrosis factor receptor 1**		

NEXT STEPS

Assay validation is expected to be completed for all candidate biomarkers by June (some of the smooth muscle marker assays may take longer due to reagent availability). At that point, Feasibility Studies will proceed with archived samples from healthy volunteers and from selected patients with vasculitides. In addition, protocols are being finalized to collect additional samples from other patient populations for upcoming Exploratory and Confirmatory studies.

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