Development of Biomarkers and Translation Strategy for Drug-Induced Vascular Injury

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Presentation Overview

• The Issue
• Introduction to DIVI
• Challenges to Risk Management and Impact to Industry
• Consortia Approaches
• Translation Strategy
• Summary
The Issue

• Small molecule for cognition in patients with schizophrenia

• 14 Day GLP toxicity studies
  – Dog- All doses clean
  – Rat- DIVI at \( \geq 100 \) mg/kg
    o Arteriopathy of hepatic arteries ranging from minimal to mild in severity.
    o No changes in hematology or clinical chemistry parameters including C-reactive protein and haptoglobin
    o No changes in HR and BP at similar doses/exposure
  – Projected Clinical Cap at 1/10th the NOAEL for DIVI = 1x Ceff AUC

• Need for biomarker to allow us monitor the finding in order to safely dose to clinical exposures > 1/10th NOAEL

Impact: Compound not progressing
Preclinical Drug-induced Vascular Injury

- Lesions develop acutely, within hours to days and is characterized histologically by one or more of the following: inflammation, necrosis, hemorrhage, and medial thickening.

- Caused by several types of drugs (PDEi, Dopamine agonists, Endothelin receptor antagonists, etc.) with differences between species (rat mesenteric arteries vs. canine coronary arteries)

- Lesions can only be detected by histopathology; there are no diagnostic or predictive circulating biomarkers

- Corresponding findings not reported in humans

- Significant challenge for pharmaceutical companies; many compounds terminated from development because of DIVI and the inability to monitor in the clinic
Preclinical DIVI: Pathology

Rat Mesenteric Arteries            Canine Coronary Arteries

Predisposed Vascular Beds Differ Among Species

Photo: Mudher Albassam
DIVI Challenges for Drug Development

• Lack of predictive and specific biomarkers for preclinical DIVI

• Uncertainty about potential for compounds causing preclinical DIVI to also be proatherogenic in humans

• Identification of an appropriate human correlate for qualifying candidate biomarkers of preclinical DIVI
  – Drug-induced vascular injury in non-clinical models differs from human clinical syndromes
  – Most drug-induced in animals not immune mediated
  – Human vasculitides? balloon angioplasty? atherogenesis?

• Lack of early *in vitro* screens for SAR or detecting target liabilities to mitigate some of these issues early
“…Arterial and venular injury is a relatively common hazard identified during nonclinical toxicity testing […]. Drugs that induce vascular lesions in animals present a safety assessment dilemma to toxicologists, physicians and regulators wishing to assess the safety of new medicines for humans. This dilemma is confounded by the gaps in our knowledge concerning pathogenesis […] and, the absence of validated nonclinical or clinical methods for monitoring vascular integrity noninvasively.”


There is an urgent need for biomarkers to detect and monitor drug induced vascular injury
DIVI is a Common Cause of Attrition

5 – 10% of development compounds cause vascular injury in preclinical toxicity studies

Some recent examples:

<table>
<thead>
<tr>
<th>Company</th>
<th>Target</th>
<th>Vascular tox observed in PreClinical (PC) /Clinical Trial (CT)</th>
<th>Issues</th>
<th>Impact</th>
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</thead>
</table>
| Company 1     | Neurological               | Hypertension / Vasospasm in CT  
Vasculitis in PC (Rat Specific)                                                              | Unknown mech.  
No monitoring parameters                                                   | Clinical development slowed by including careful monitoring. Negative impacts on submission. |
| Company 1     | PDE3/4                     | Vasculitis in PC (Rat Specific)                                                                  | Unknown mech.  
No monitoring parameters                                                   | Clinical trial stopped with this target                                   |
| Company 2     | PDE4 inhibitor (oral)      | Vasculitis in PC (mesenteric artery-rat; coronary artery-monkey)                                | Unknown mech.  
No monitoring parameters                                                   | Clinical doses were limited due to 2x multiple for DIVI. Clinical trial stopped with this target |
| Company 3     | Anti-inflammatory, biologic | Vasculitis in PC (Primate specific)                                                              | Unknown mechanism;  
No monitoring parameters                                                    | Terminated preclinically                                                  |
| Company 4     | Anti-inflammatory small molecule | Vasculitis in PC (Rat Specific)                                                                  | Unknown mechanism;  
No monitoring parameters                                                    | Project team no longer active                                             |
• Critical Path Initiative – Predictive Safety Testing Consortium (PSTC)
  – Kidney, liver, vascular, muscle (skeletal, cardiac), carcinogenicity
  – Preclinical and clinical translation focus
  – Funding from public/private foundations (staff and infrastructure) and industry (membership fee + in-kind contributions)

• Health and Environmental Sciences Institute (HESI)
  – Kidney, cardiovascular (troponins) – preclinical focus
  – Industry funded membership fee plus in-kind contributions

• Innovative Medicines Initiative (IMI) – SAFE-T Consortium
  – Kidney, liver, vascular injury
  – Clinical translation focus
  – IMI – EU government provides 50% funding which supports academic partners. Industry matches EU funding with in-kind support
• Initial focus on qualifying preclinical biomarkers of vascular injury

• Developing assays to monitor endothelial and smooth muscle cell injury, and vascular inflammation
  – Obtained proof of concept for 10 markers (5 inflammatory, 4 endothelial, and 1 smooth muscle)
  – Assay development for 11 additional markers underway (smooth muscle and endothelial)
  – Completed 10 rat studies to provide samples for BM proof of concept studies and qualification efforts (with at least 6 more planned for 2012).

• Will submit FDA letter initiating Biomarker Qualification process in 2012

• Strong synergy with IMI SAFE-T around translation
Clinical Qualification Strategy

• Our challenge is to design a qualification strategy for biomarkers of a preclinical toxicity that has no direct clinical correlate
  – In contrast to DILI (e.g., APAP and ALT) or DIKI (e.g., cisplatin and BUN/creatinine), there is no gold standard to perform “standard” ROC analyses for DIVI

• Most currently used biomarkers of vascular inflammation in the clinic are non-specific
  – Include markers of endothelial and smooth muscle cell damage
  – Result is that a panel of biomarkers is likely necessary

• Candidate biomarkers selected on basis of both preclinical and clinical association with vascular damage
  – Thus, a forward and reverse translation strategy is necessary
  – Interaction between PSTC and SAFE-T is important

• Overall, our strategy is to use patient populations presenting disease or drug-induced vascular damage

*We hypothesize that similar histopathology between preclinical DIVI and vascular injury/disease in humans will lead to overlapping biomarker signatures*
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, and the other members of SAFE-T “Workpackage 4” (WP4*)

ABSTRACT

Drug-induced vascular injury (DIVI) is a serious complication involving organ damage and death in patients. Current diagnostic methods are insufficiently sensitive to identify DIVI at preclinical stages. Thus, there is a need for biomarkers and biomarker-based tools to detect and predict DIVI at early stages.

Preclinical animal studies are crucial to identify DIVI mechanisms and validate potential biomarkers. However, preclinical results do not always translate into clinical settings due to the differences in vascular anatomy and physiology between animals and humans. Therefore, the focus is on developing a DEC (Dose Evaluation Criteria) that can predict DIVI in humans with a similar risk as preclinical studies.

METHODS

We reviewed the literature on DIVI in preclinical and clinical settings. We analyzed the current diagnostic tools and potential biomarkers for DIVI. We also discussed the DEC criteria and the need for a more accurate translation from preclinical to clinical settings.

RESULTS

We found that early detection of DIVI is crucial to prevent severe complications and mortality. Preclinical studies have revealed the involvement of various mechanisms in DIVI, such as endothelial dysfunction, inflammation, and thrombosis. However, the translation of these findings to humans has been challenging.

DISCUSSION

We propose a novel DEC that integrates clinical and preclinical data to improve the translation of DIVI biomarkers. The DEC considers factors such as the dose, route of administration, and duration of treatment to predict early signs of DIVI. This approach can help identify patients at risk of DIVI and guide preventive strategies.

CONCLUSION

The development of effective biomarkers and DEC criteria is essential to improve the translation of DIVI in preclinical and clinical settings. Further research is needed to validate these tools and optimize their implementation in clinical practice.

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Biomarkers of endothelial cell activation: Candidate markers for drug-induced vasculitis in patients or drug-induced vascular injury in animals

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ABSTRACT

There is a pressing need for vascular biomarkers for studies of drug-induced vasculitis in patients and drug-induced vascular injury (DIVI) in animals. We previously reviewed a variety of candidate biomarkers of endothelial cell (EC) activation (Zhang et al., 2010). Now we update information on EC activation biomarkers from animal data on DIVI and clinical data of vasculitic patients, particularly patients with primary antineutrophil cytoplasmic autoantibody (ANCA)-associated small vessel vasculitis (primary AAVs), including Wegener’s granulomatosis, microscopic polyangiitis, Churg–Strauss syndrome and necrotizing crescentic glomerulonephritis. Drug-associated ANCA-positive small vessel vasculitis (drug-AAV) can closely resemble primary AAVs, suggesting the large overlap between primary idiopathic systemic vasculitis and drug-induced vasculitis. AAVs in patients and DIVI in animals vary considerably; however, there is close resemblance between AAVs and DIVI in some respects: (1) the immunopathogenetic mechanisms (activation of primed neutrophils, ECs and T cells by ANCA in patients and activation of ECs, mast cells, and macrophages by drugs in animals); (2) the morphologic changes (fibrinoid necrosis of the vessel wall and neutrophilic infiltration); (3) the preferable sites (small arteries, arterioles, capillaries and venules); and (4) elevation of vascular biomarkers suggestive of an endothelial origin. The present review discusses soluble and cell component biomarkers and provides a rationale for the potential utility of EC activation biomarkers in nonclinical and clinical studies during new drug development. Further investigation, however, is needed to assess their potential utility.
IMI SAFE-T Consortium

Members: 140 participants from 11 Pharma, 4 SME and 6 academic centres
Duration: 5 years (started June 09)
Budget: 38 M€
Funding: Budget 35.7 million € (13.9 million € as IMI-JU contribution, 17.6 million € as EFPIA contribution)
Governance structure: Steering Committee, Scientific Advisory Committee, Ethics Committee, IP Committee
Project coordinator: Michael Merz (Novartis)
Scientific coordinator: Ina Schuppe Koistinen (AZ)
Website: www.imi-safe-t.eu/

Pharma:
• Almirall
• Amgen
• Astra Zeneca
• Bayer Schering Pharma AG
• Boehringer Ingelheim
• Eli Lilly
• GlaxoSmithKline
• Novartis
• Hoffmann La Roche
• Pfizer
• Sanofi aventis

External Advisors:
• EMA
• FDA

Academic:
• Barcelona Cardiovascular Research Center
• Charité Hospital
• Groupe d’Etudes et de Recherches en Médecine Interne et Maladies Infectieuses - APHP
• Groupe Hospitalier Pitié Salpêtrière - APHP
• Natural and Medical Sciences Institute
• Tel-Aviv (Souraski) Medical Center

Small-Medium Enterprises (SMEs):
• Argutus Medical Limited
• Experimental & Diagnostic Immunology GmbH
• Firalis SAS
• Interface Europe

Collaborators:
• Predictive Safety Testing Consortium
• University of Malaga/ Spanish DILI Registry
• University of Liverpool/Centre for Drug Safety Sciences
SAFE-T Structure and Deliverables

- Evidence-based decision making
- More reliable causality assessment
- Better mechanistic understanding
- Safer translation to clinical development
- Earlier and more specific signal detection
- Enhanced clinical monitoring
  - Improved patient safety
  - Reduced attrition rates
  - Accelerated and safer approval of innovative medicines
Parallel Reverse/Forward Translation

Biomarker candidates of preclinical DIVI

Preclinical marker

Exploratory studies

Confirmatory studies

Preclinical qualification studies

Clinical marker candidates

Biomarker candidates of damaged vessels in man

Damage from DIVI-drugs

Damage from drugs, immune-mediated diseases, atherosclerosis, radiation, etc.
Populations of Interest

**Males**
- Vascular disorders
  - Large vessel vasculitides
    - Behçet's disease
    - Takayasu's disease
  - Small/Medium vessel vasculitides
    - Mixed cryoglobulinemia
    - Buerger's disease
    - Primary Sjögren's syndrome
  - Drug induced vasculitides
- Non vascular conditions
  - Acute infection
  - CH hepatitis
  - Cirrhosis
  - Active cancer
  - Multiple sclerosis
  - Rheumatoid arthritis
  - Transplant rejection

**Females**
- Vascular disorders
  - Hypertension
  - Dyslipidemia CAD, MI
  - Type 2 diabetes
  - Drug induced vasculitides
- Non vascular conditions
  - Rheumatoid arthritis
  - Transplant rejection
  - Active cancer
  - Multiple sclerosis
  - Cirrhosis
  - Acute infection
  - CH hepatitis

**Healthy subjects**
- Non vascular conditions
  - C hepatitis
  - Acute infection
  - CH hepatitis
  - Cirrhosis
  - Active cancer
  - Multiple sclerosis
  - Rheumatoid arthritis
  - Transplant rejection

**Other parameters**
- (age, diet, exercise, BMI, ethnicity)
Endothelial and smooth muscle injury as well as inflammation are integral morphologic features of preclinical DIVI.

- Smooth muscle damage: fibrinoid necrosis
- Endothelial cell damage: activation
- Mixed perivascular inflammatory cell infiltrate
Candidate Biomarkers (SAFE-T, PSTC)

**Common markers**
- ACTA2
- CALD1
- SMTN
- TAGLN
- CNN1
- CAV1
- EDN1
- EMPs
- ESM1
- THSP1
- ICAM
- VEGF
- vWFpp
- ELAM1

**SAFE-T only**
- VCAM
- THBD
- vWF
- ACE
- SELP
- CCL19
- CXCL10
- CXCL11
- CXCL12
- CXCL9
- TNFRS1
- IL8
- TGFb
- CCL3
- CRP
- CLU

**VIWG only**
- Angpt2
- Aoc3
- Eln
- Fndc3
- Hdac8
- PPIA
- AGP
- C3a
- Cxcl1
- Lcn2
- Myh11

**Legend**
- SM marker
- Inflammatory marker
- Endothelial marker
Biomarker Translation?

ANIMALS WITH DRUG-INDUCED VASCULAR INJURY IN SMALL VESSELS
CRP, IL-6, IL-8, α2-macroglobulin, sTM, svWF
α1-acid glycoprotein, α-fetoprotein, β-fibrinogen, β2-glycoprotein, heptoglobin, hemopexin
apolipoproteins, nitrite vascular endothelial growth factor, tissue inhibitor of metalloproteinase-1

PATIENTS WITH SMALL VESSEL VASCULITIS
CRP, sIL-6, sIL-8, α2-macroglobulin, sTM, sE-selectin, sICAM-1, sVCAM-1, svWF, CECs, EMPs
sIL-1ra, PTX3, sTPA, TNF-α, p55TNF-α-R, MCP, sTF

PATIENTS WITH MEDIUM VESSEL VASCULITIS
CRP, sIL-6, sTM, sE-selectin, sICAM-1, sVCAM-1
svWF, CECs, EMPs, SAA

PATIENTS WITH LARGE VESSEL VASCULITIS
sIL-6, sTM, sE-selectin, sICAM-1, sVCAM-1
svWF, CECs, EMPs, RANTES

Zhang et al., 2011
Regulatory Interactions

• VXDS meeting held on October 6, 2010
  – Requested by SAFE-T
  – Attended by FDA and EMA
  – Meeting objective:
    o To obtain the agencies’ feedback and guidance on the exploratory-phase qualification strategy for translational safety biomarkers of drug-induced vascular injury (DIVI).
  – Feedback was generally positive, with numerous suggestions incorporated in revised SAFE-T strategy plan

• PSTC VIWG will submit FDA letter initiating Biomarker Qualification process in 2012
• Preclinical DIVI remains a significant drug development issue for the Pharmaceutical industry.

• While histopathology remains the gold standard for detection of vascular injury, much progress has been made identifying potential markers of DIVI.

• Clinical qualification of DIVI biomarkers has some unique challenges

• The coordinated effort enabled by consortia has helped prioritize markers, lower overall cost, and speed biomarker validation and qualification.

• Alternative biomarkers of vascular injury, including endothelial microparticles, imaging, micro RNAs, and localized blood flow measurements, are promising and may be more easily translatable.

• Continued dialog with regulators is key to success; need to define the “Context of Use” for translatable DIVI biomarkers