



**Engineered Biomarkers for Non-
Invasive**

**Prediction of DNA damage and
Oxidative Stress *In Vivo***

CXR's *in vivo* ToxReporter systems

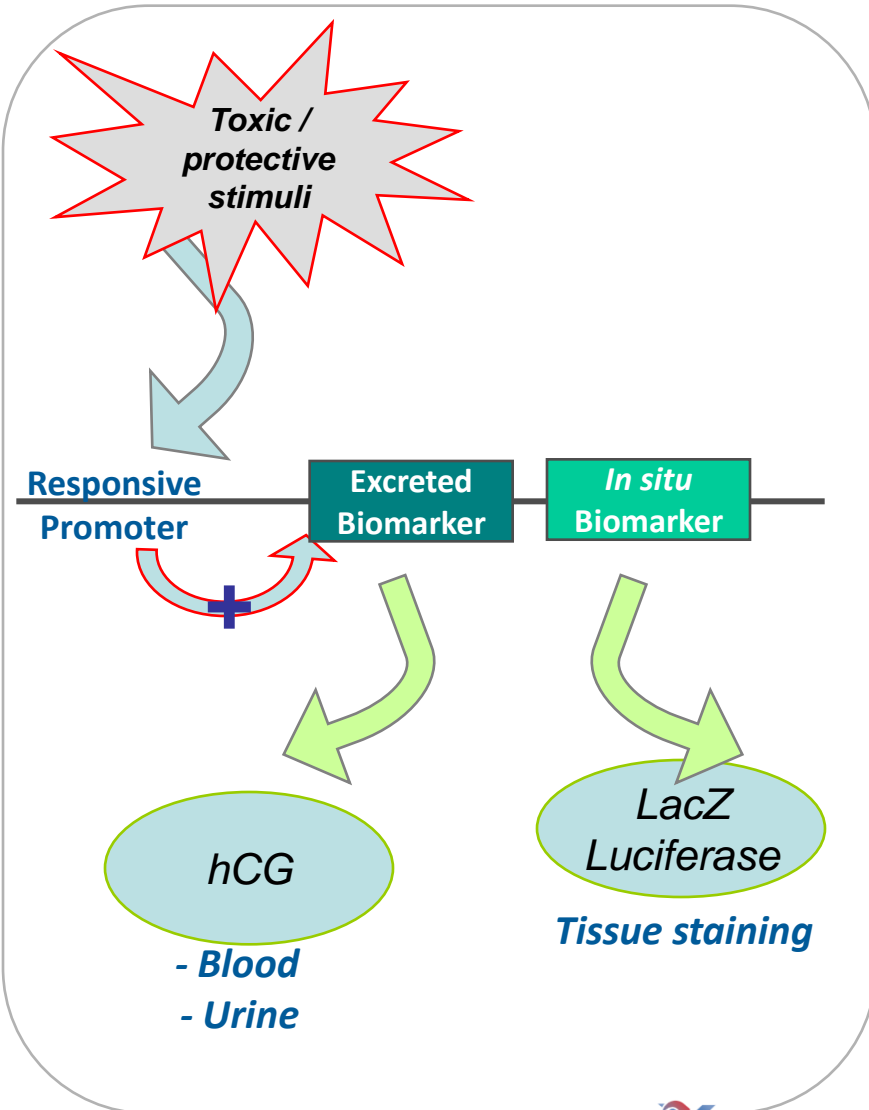
In vivo models for the rapid & informative assessment of mechanisms of toxicity

- Two characterised models:

- **Oxidative Stress (HMOX)**: Haem Oxygenase I reporter
- **DNA Damage (WAZ)**: Cyclin-dependent kinase inhibitor-1A (p21^{waf1}) reporter

- Uses:

- Accurate read-out of temporal and dose response to drug candidate during experiment
- Demonstrate *in vivo* effect prior to pathological change
- Localisation of mechanistic response at end of experiment

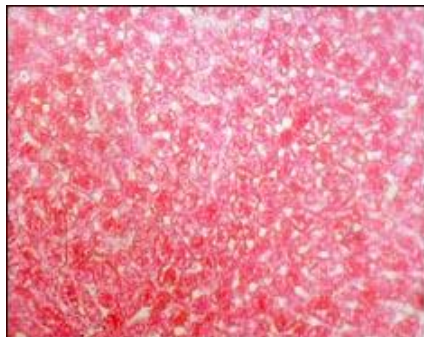


Hmox1 Reporter Mice

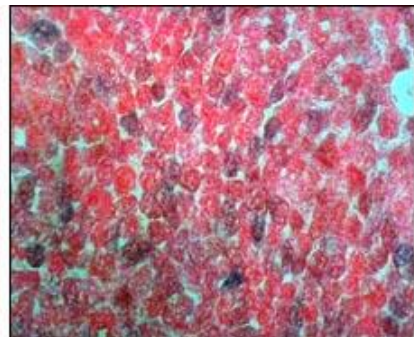
- Uses the promoter from the Haem oxygenase 1 (Hmox1) gene
- Hmox1:
 - An inducible isoform of the enzyme involved in the degradation of haem to biliverdin IX α , free divalent iron and carbon monoxide
 - Induced through stress responses e.g. Hypoxia, inflammation, LPS, UVA radiation, oxidising agents & heavy metals (Morse and Choi, 2002)
- Validation data generated with known inducers of Hmox-1
 - Haemin
 - Acetaminophen
 - CdCl₂

Oxidative stress in liver caused by CdCl₂ shows a dose-response and can be measured in urine

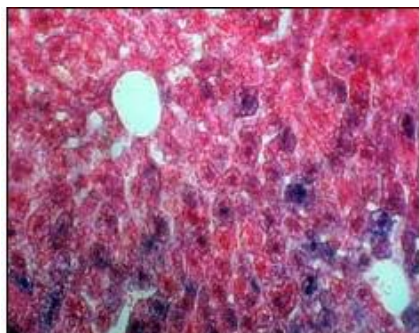
Control



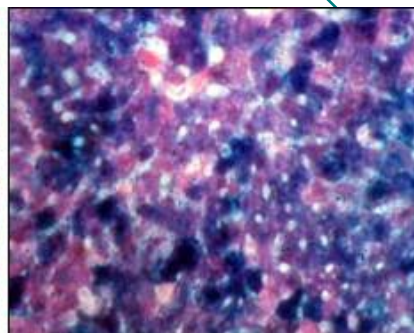
2 mg/kg



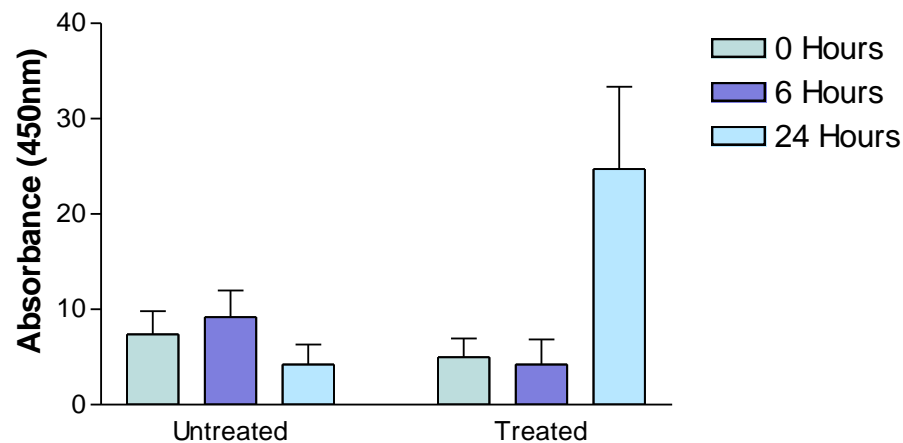
4 mg/kg



8 mg/kg



Urinary hCG Marker of Hmox-1

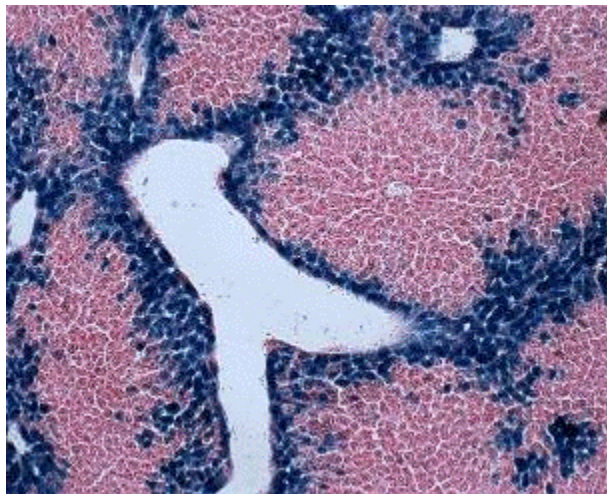


Oxidative stress caused by CdCl₂ is indicated by LacZ expression in the liver of mice transgenic for Hmox-1, and pattern follows a dose response

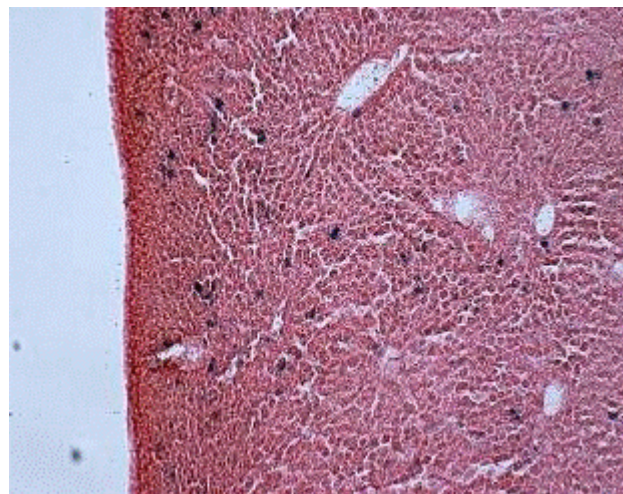
Urinary biomarker of oxidative stress elevated before ALT levels – non-invasive measurement

The *in situ* marker allows exact site of oxidative stress response to be identified

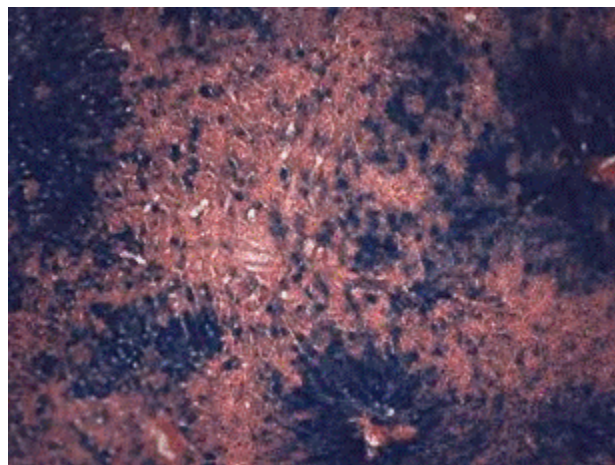
HOD Mouse 300mg/kg Acetaminophen



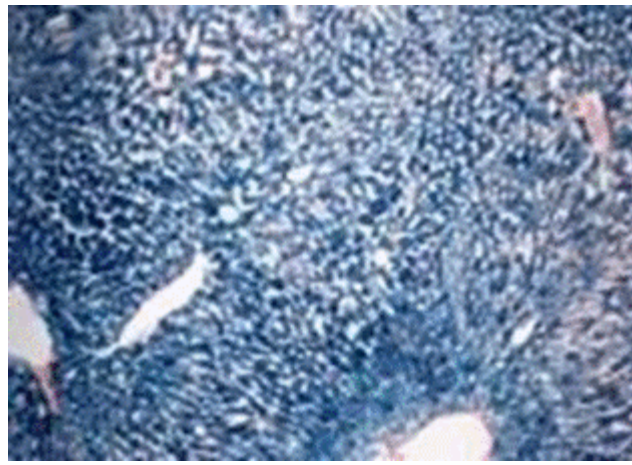
HOD Mouse 200mg/kg Diclofenac



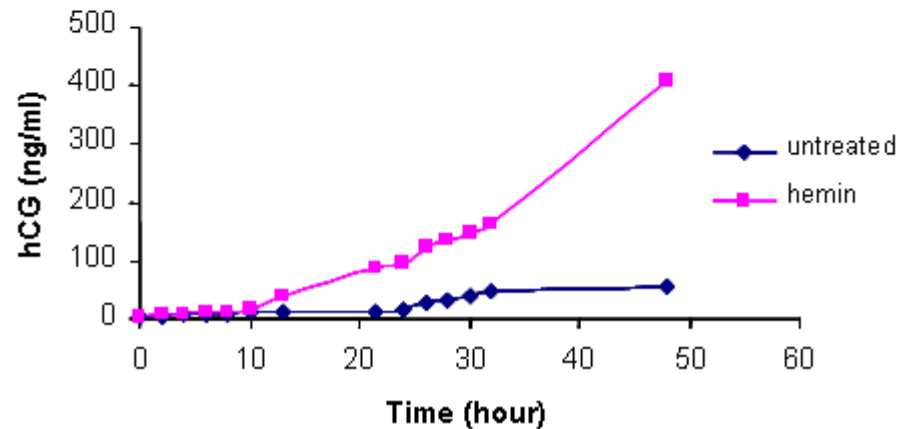
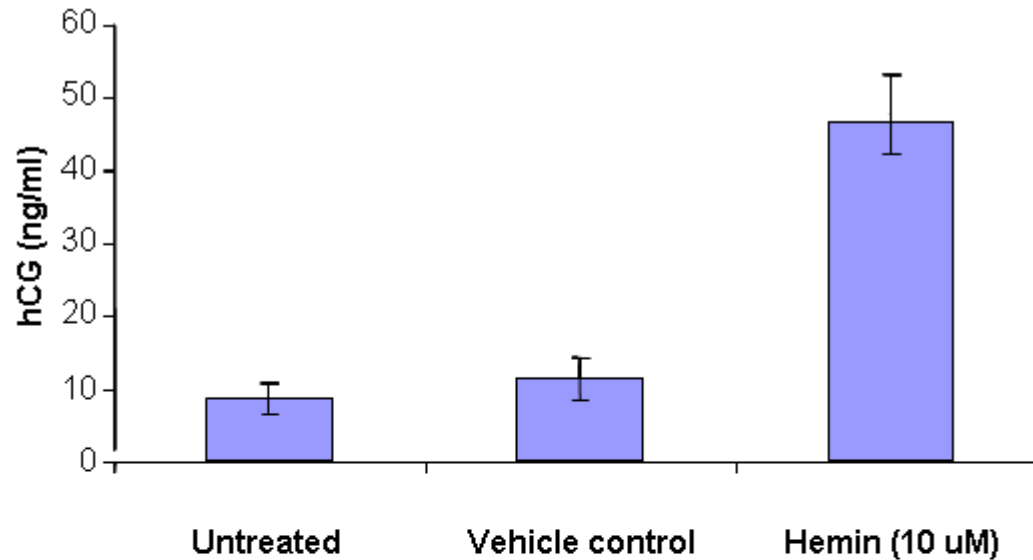
HOZ Mouse 65mg/kg Hemin



HOZ Mouse 4mg/kg Cadmium Chloride



The excreted marker shows the temporal induction of the stress response



WAZ Reporter Mice

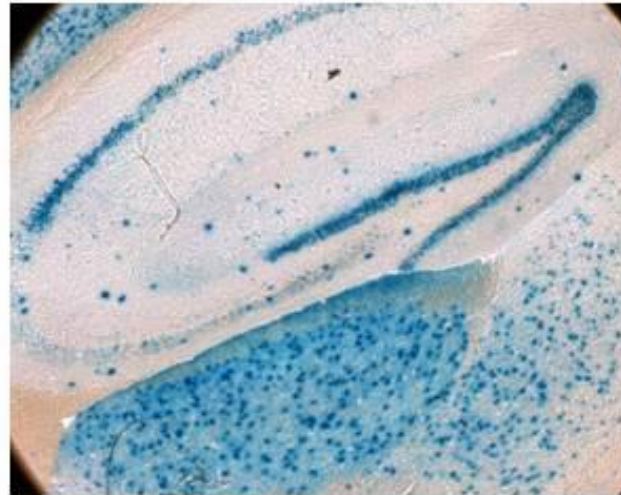
- Utilises the promoter from the p21^{WAF1} gene and the *in situ* LacZ reporter protein
- p21/Waf1:
 - Essential downstream mediator of p53-dependent G1/S phase checkpoint control
 - Belongs to the Cip/Kip family of CKIs (p21Cip1/WAF1, p27Kip1, p57Kip2), which share significant sequence homology in their amino-terminal portions and recognize a broad, but not identical range of cyclin/CDK targets (Sherr and Roberts, 1999).
- Validation data generated with known inducers of p21:
 - Etoposide
 - LPS
 - HgCl₂

In Situ Marker - p21 Induction by chemotherapeutic Etoposide

Untreated

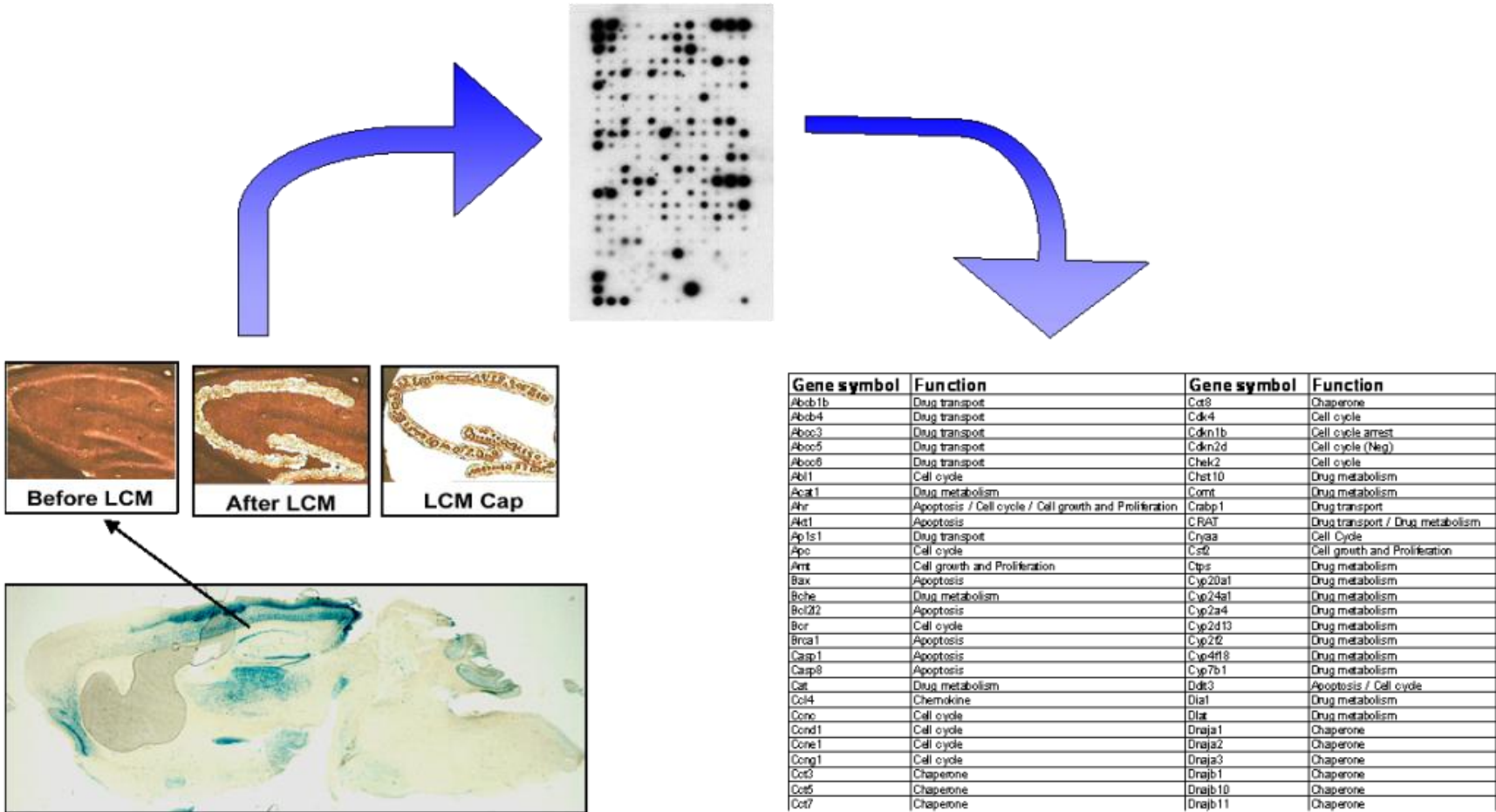


Etoposide
Treated



Mouse
Brain

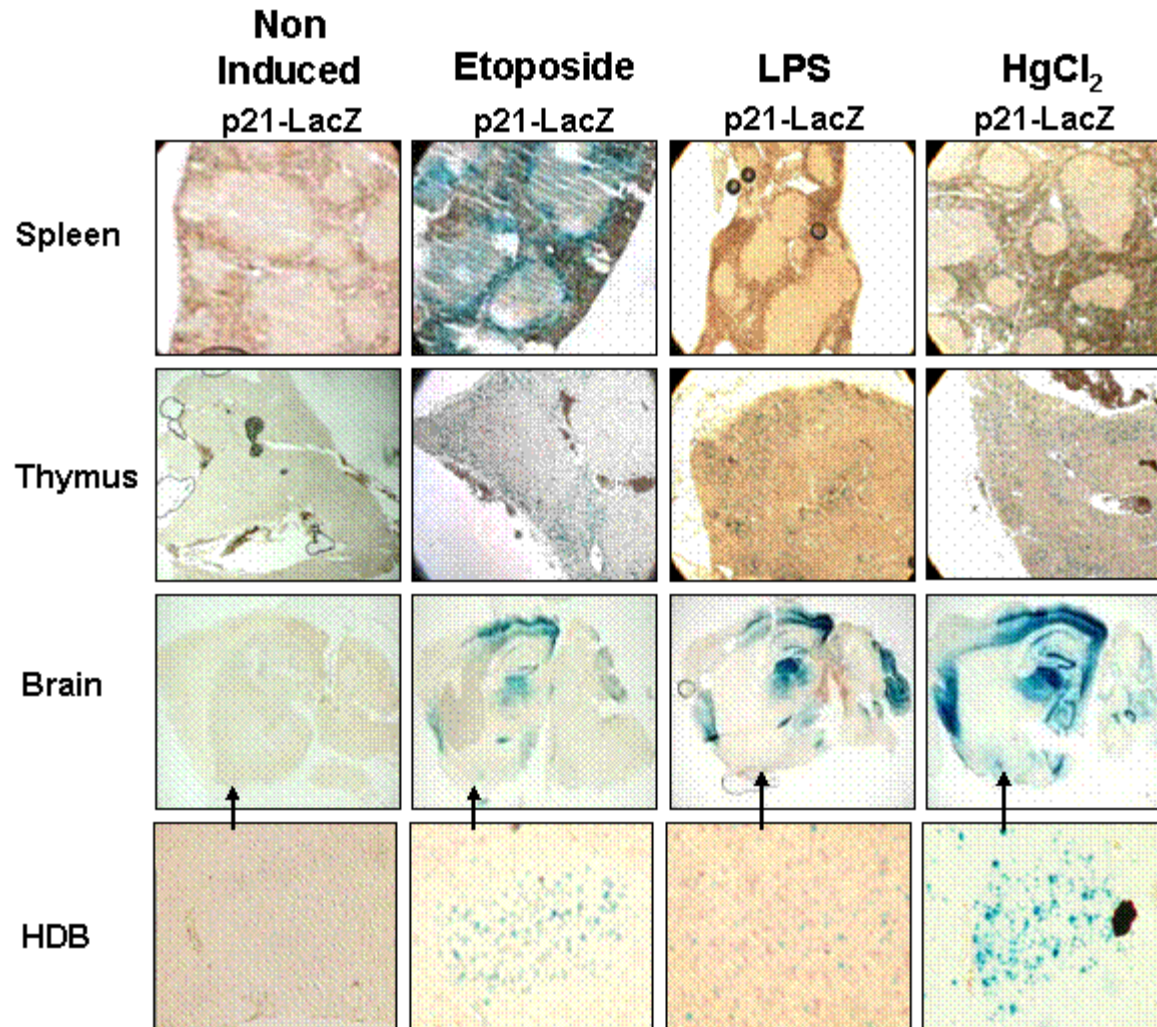
Laser Microdissection of p21-LacZ area induced by Etoposide & Microarray Analysis



WAZ reporter mice exposed to etoposide . After 24h brain sections were stained for *lacZ* and affected areas removed by laser capture microdissection (LCM). Subsequent to this microarray analysis was carried out.

WAZ Reporter Mice Validation Data

Induction of p21-LacZ transgene with various inducers



Key publication and contact:

p21-LacZ reporter mice reflect p53-dependent toxic insult

Douglas B. Vasey, C. Roland Wolf, Thomas MacArtney,
Ken Brown, C. Bruce A. Whitelaw

Toxicology and Applied Pharmacology, 227 (2008), 440–450

Please contact Prof Roland Wolf to discuss interest in the Reporter Mice models

Email: rolandwolf@cxrbiosciences.com

Tel: +44 1382 432163



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