BHF Centre of Research Excellence

British Heart Foundation

THE UNIVERSITY OF EDINBURGH

NPIS
National Poisons Information Service

EdinClinTox
Edinburgh Clinical Toxicology

Paracetamol and precision toxicology

Dr James Dear
Reader in Clinical Pharmacology

Who am I?

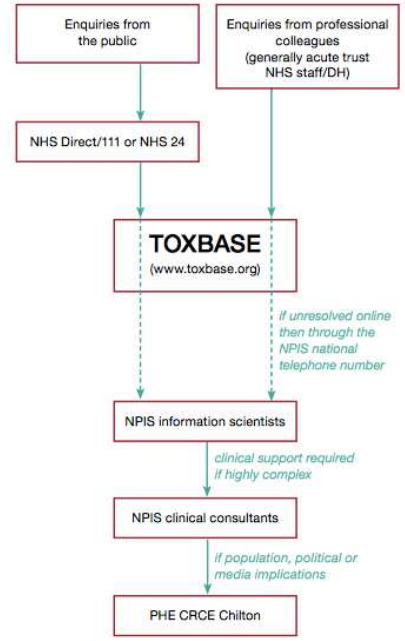
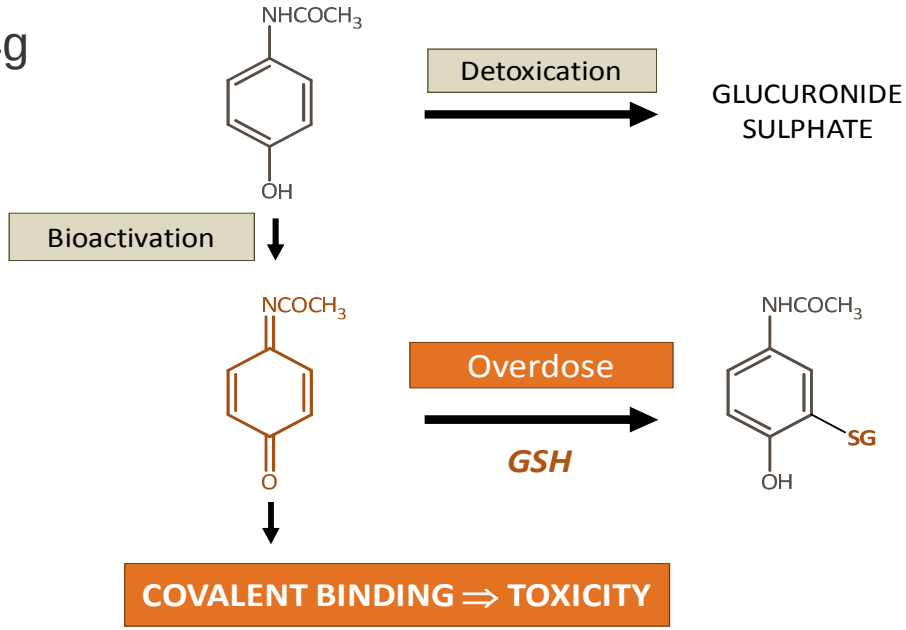
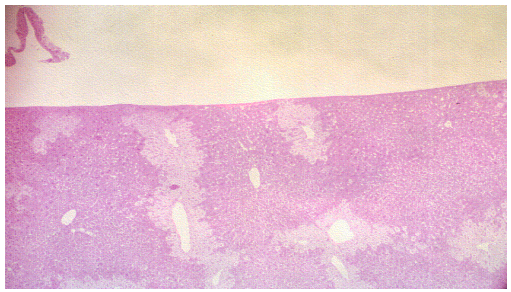


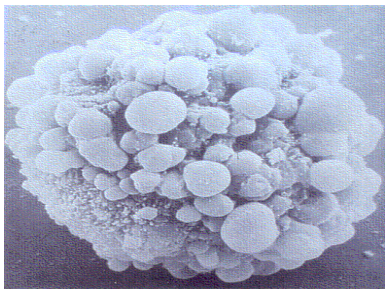
FIGURE 2.1 How poisons enquiries are answered

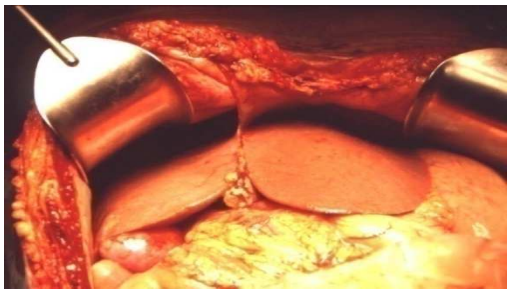
Acetaminophen (paracetamol)

- Recommended dose - 4g. Toxic dose >4g
- Most common form DILI in US & UK
- Treatment with acetylcysteine
- Cannot design out toxicity
- Cleary defined chemical insult
- Multiple mechanisms reported – apoptosis, necrosis, mitochondrial dysfunction, inflammation

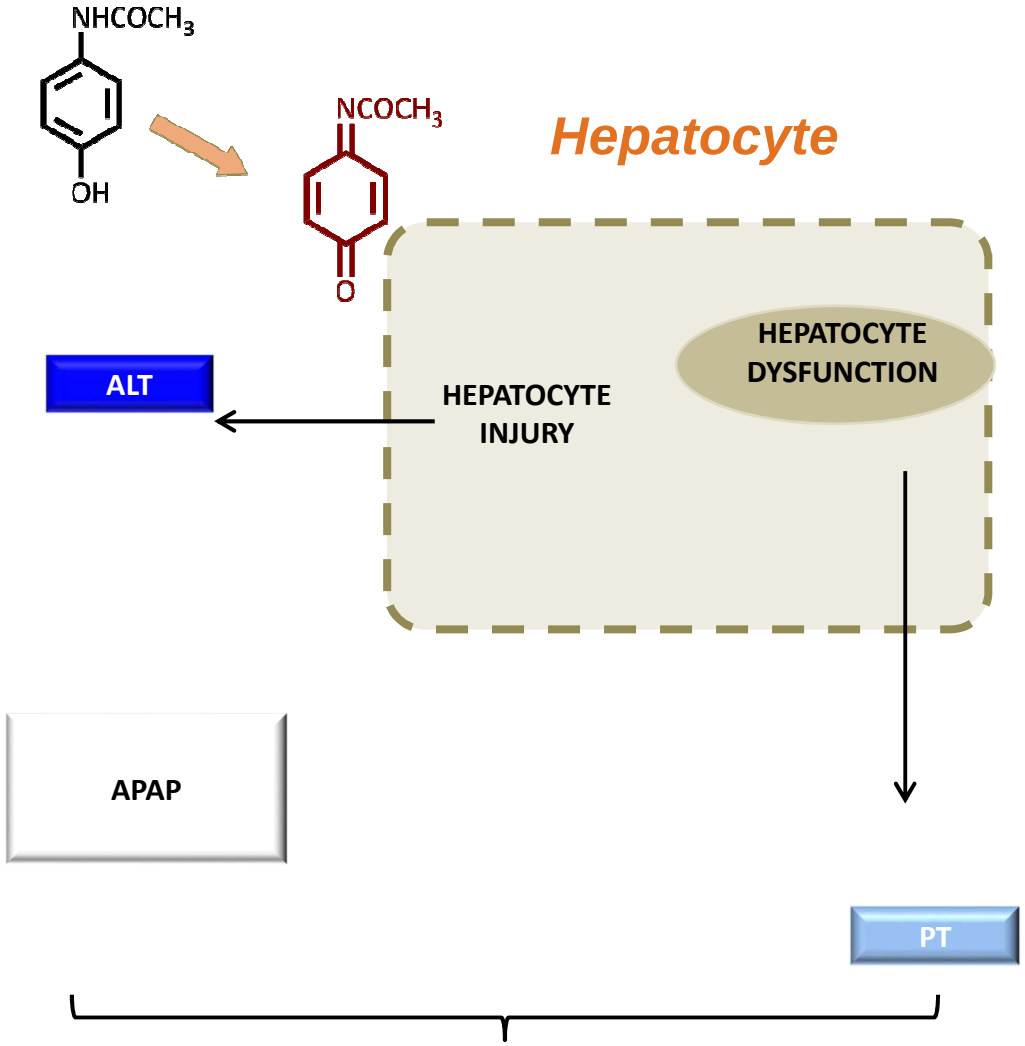






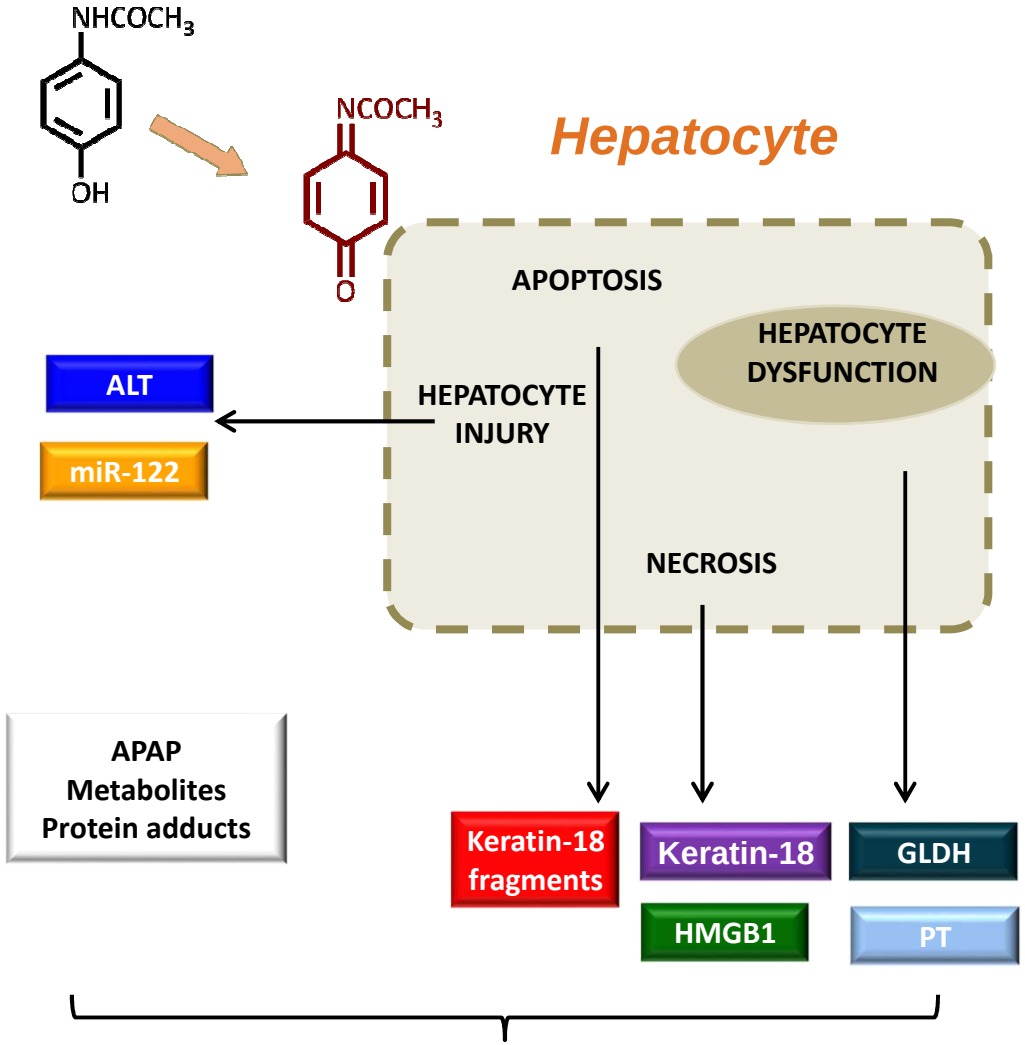


Edinburgh and Liverpool biomarker panel



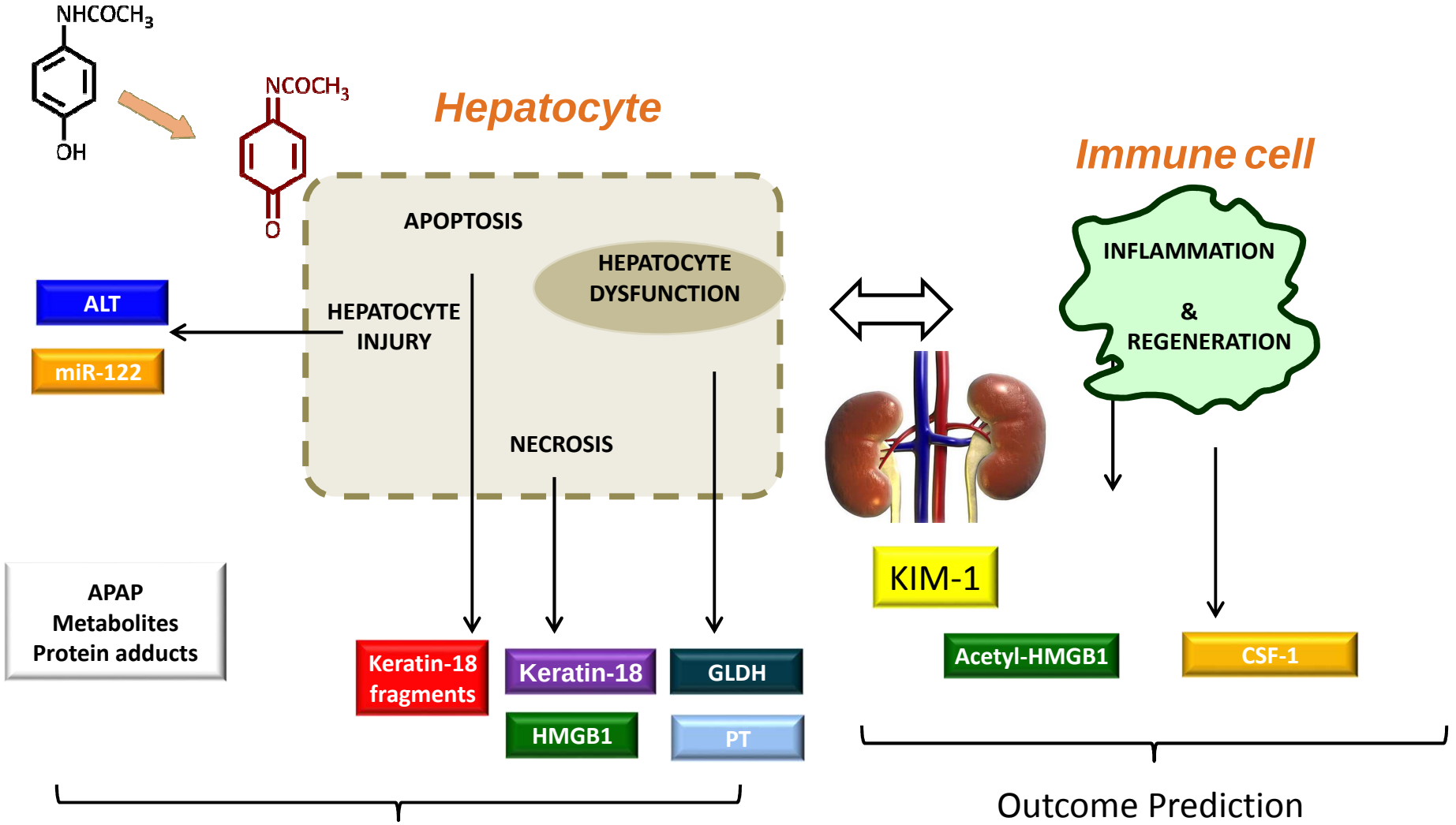
Sensitive and mechanistic diagnostics of ALF

Edinburgh and Liverpool biomarker panel



Sensitive and mechanistic diagnostics of ALF

Edinburgh and Liverpool biomarker panel



PARACETAMOL QUESTION 1:

Who needs treatment?

- Improved early patient stratification

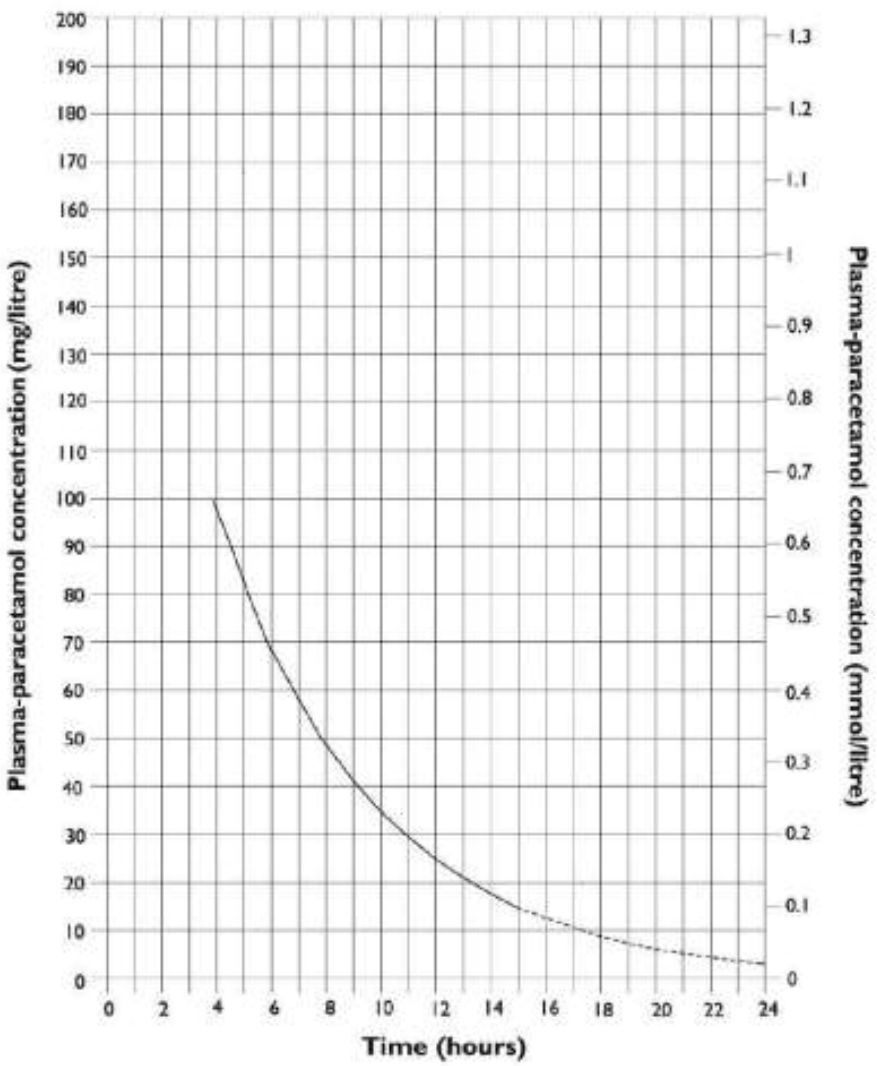
Why paracetamol?

Hospital Episode Statistics

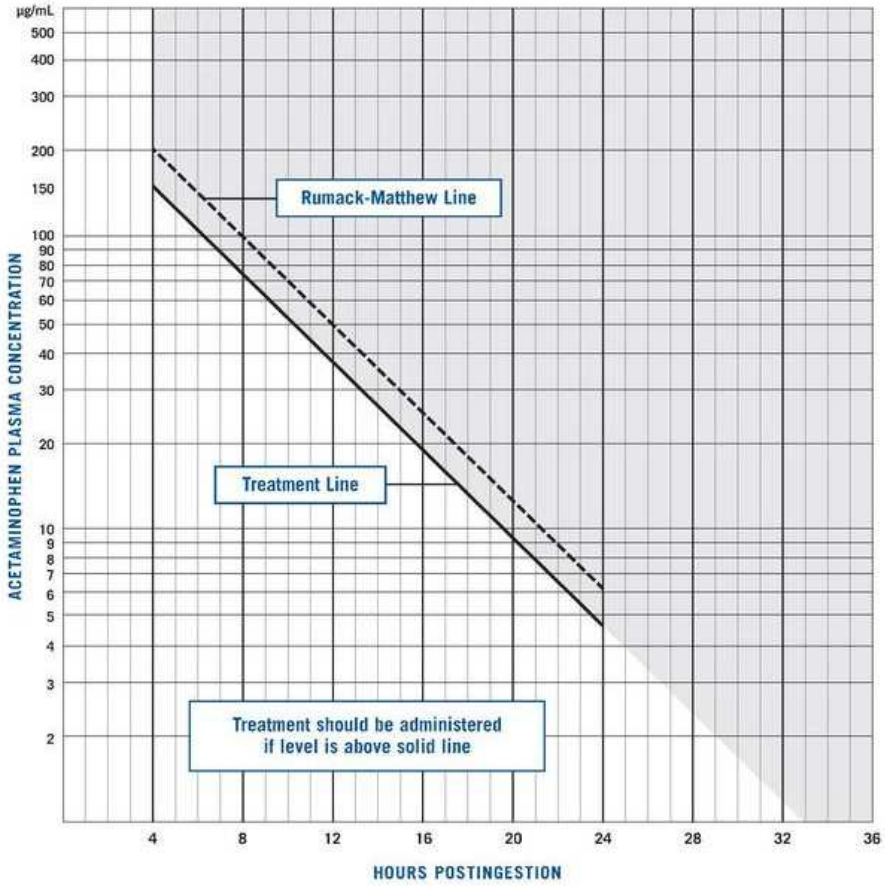
Reason for admission	Emergency admissions (England)
Paracetamol	**41,778**
Fracture of neck of femur	42,616
Congestive heart failure	37,148
Acute myocardial infarction	26,967
Acute exacerbation of COPD	44,969

Risk assessment – paracetamol concentration

UK



USA



microRNA-122

Centre for
Drug Safety Science

Circulating MicroRNAs as Potential Markers of Human Drug-Induced Liver Injury

Philip J. Starkey Lewis,[1*] James Dear,[2*] Vivien Platt,[1] Kenneth J. Simpson,[3] Darren G.N. Craig,[3] Daniel J. Antoine,[1] Neil S. French,[1] Neeraj Dhaun,[4] David J. Webb,[4] Eithne M. Costello,[5] John P. Neoptolemos,[5] Jonathan Moggs,[6†] Chris E. Goldring,[1†] and B. Kevin Park[1†]

Hepatology 2011

Kevin Park

Philip Starkey Lewis

Dan Antoine

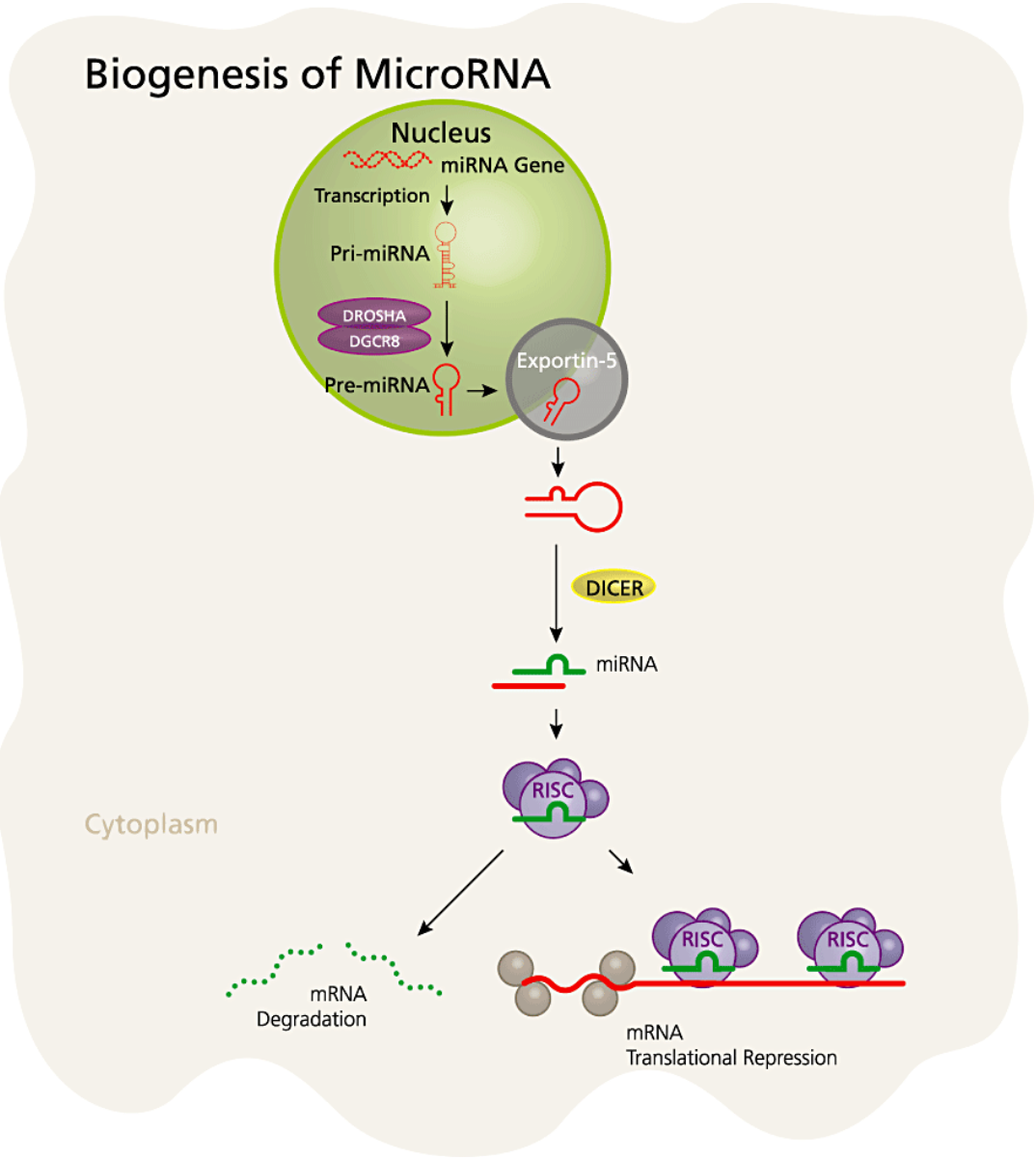
Mechanistic Biomarkers Provide Early and Sensitive Detection of Acetaminophen-Induced Acute Liver Injury at First Presentation to Hospital

Daniel J. Antoine,[1*] James W. Dear,[2,3*] Philip Starkey-Lewis,[1*] Vivien Platt,[1] Judy Coyle,[4] Moyra Masson,[4] Ruben H. Thanacoody,[5] Alasdair J. Gray,[4] David J. Webb,[2,3] Jonathan G. Moggs,[6] D. Nicholas Bateman,[2] Christopher E. Goldring,[1] and B. Kevin Park[1]

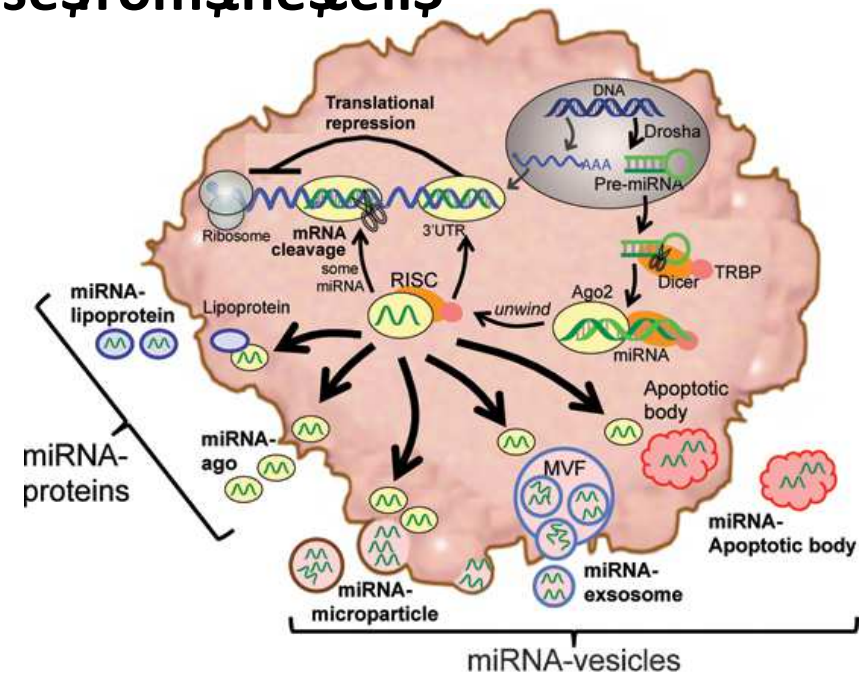
Hepatology 2013

miR-122 is specific for the hepatocyte
And detectable earlier than ALT -sensitive

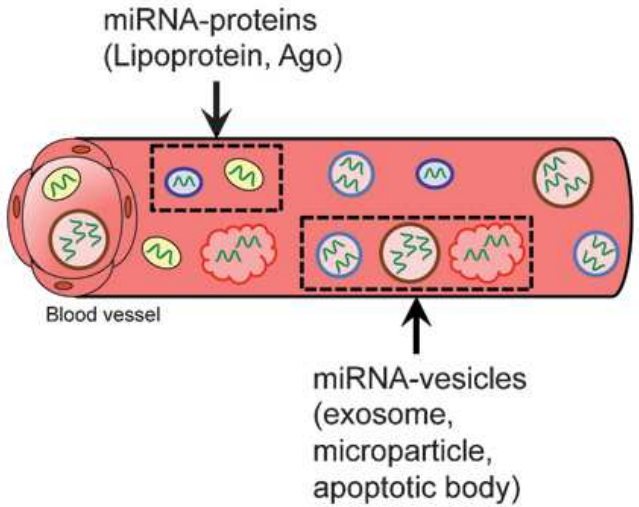
microRNA


Biogenesis of MicroRNA
Nucleus
miRNA Gene
Transcription
Pri-miRNA
DROSHA
DGCR8
Pre-miRNA
Exportin-5
DICER
miRNA
RISC
Cytoplasm
RISC
RISC
mRNA
Degradation
mRNA
Translational Repression

microRNA$release$fromthecell$



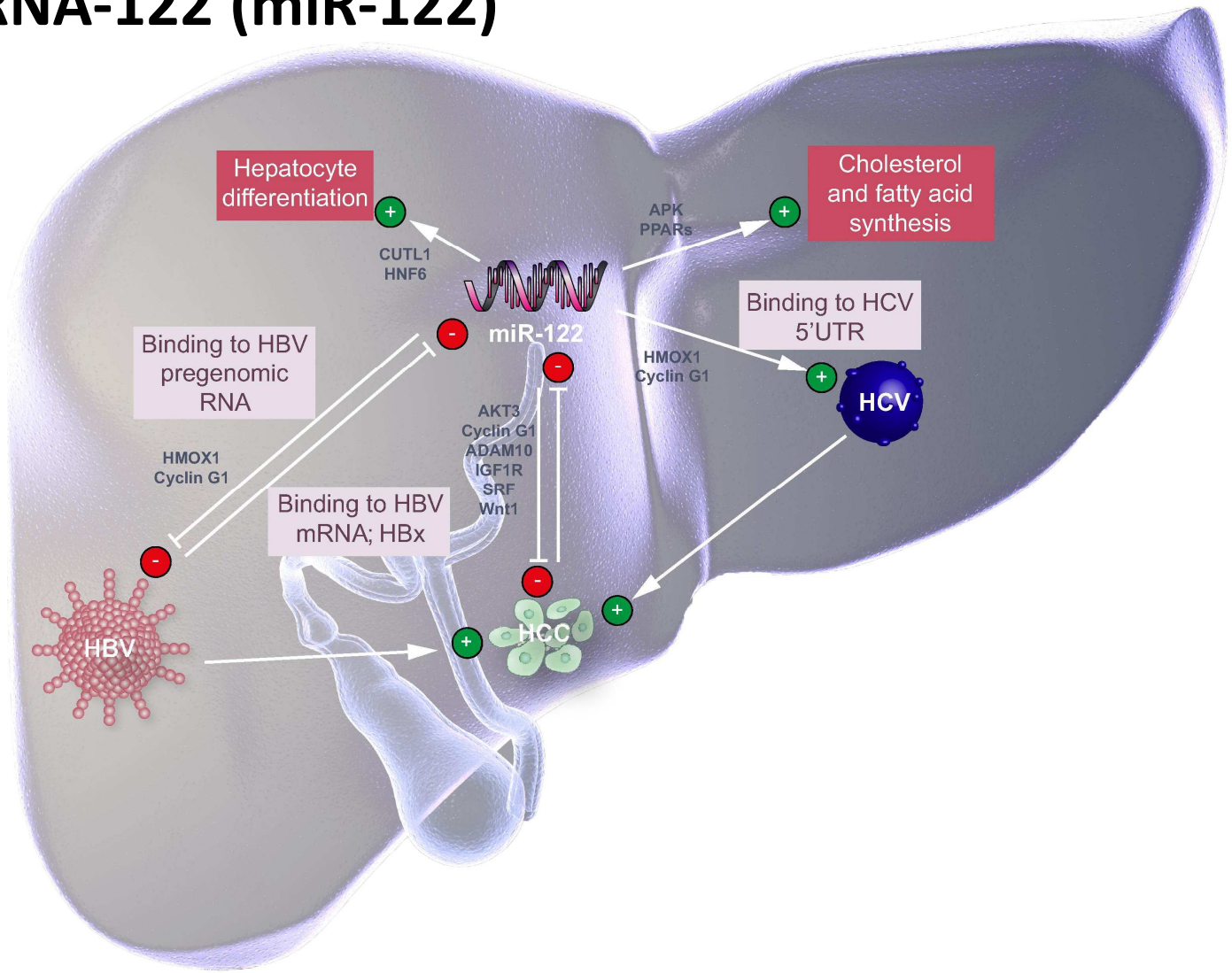
Hepatocyte



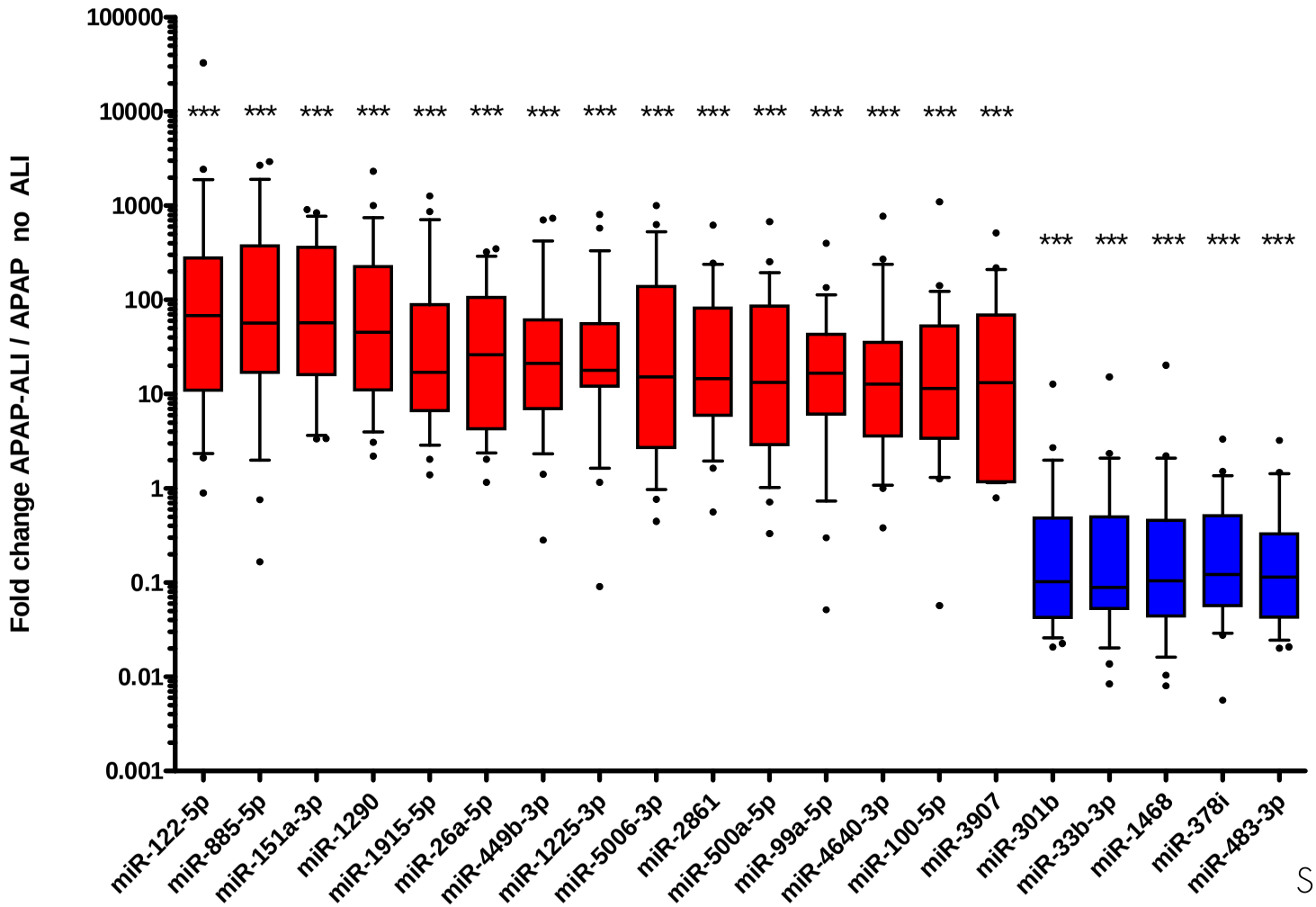
Blood$vessel

Biomarker

microRNA-122 (miR-122)



miR-122 in paracetamol DILI



SCIENTIFIC REPORTS

OPEN

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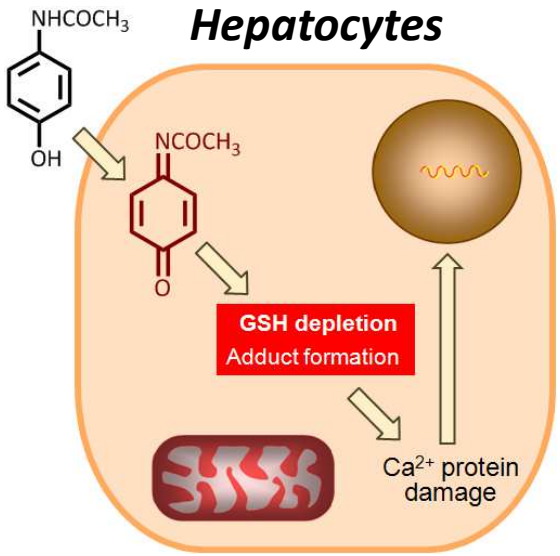
Published: 22 October 2015

Comprehensive microRNA profiling in acetaminophen toxicity identifies novel circulating biomarkers for human liver and kidney injury

A. D. B. Vliegenthart[1], J. M. Shaffer[1], J. I. Clarke[1], L. E. J. Peeters[1], A. Caporali[1], D. N. Bateman[2], D. M. Wood[2,3], P. I. Dargan[2,3], D. G. Craig[2], J. K. Moore[2], A. I. Thompson[2], N. C. Henderson[1], D. J. Webb[1], J. Sharkey[1], D. J. Antoine[1], B. K. Park[1], M. A. Bailey[1], E. Lader[4], K. J. Simpson[5] & J. W. Dear[1]

Biomarker

Mechanism of Cell Death - Biomarkers



- **Cytokeratin 18 – abundant in hepatocytes**
- **Is cleared by caspases**
- **Fragment released into plasma**

- **HMGB1 - released by necrotic cells**

The Journal of Clinical Investigation RESEARCH ARTICLE

The HMGB1/RAGE axis triggers neutrophil-mediated injury amplification following necrosis

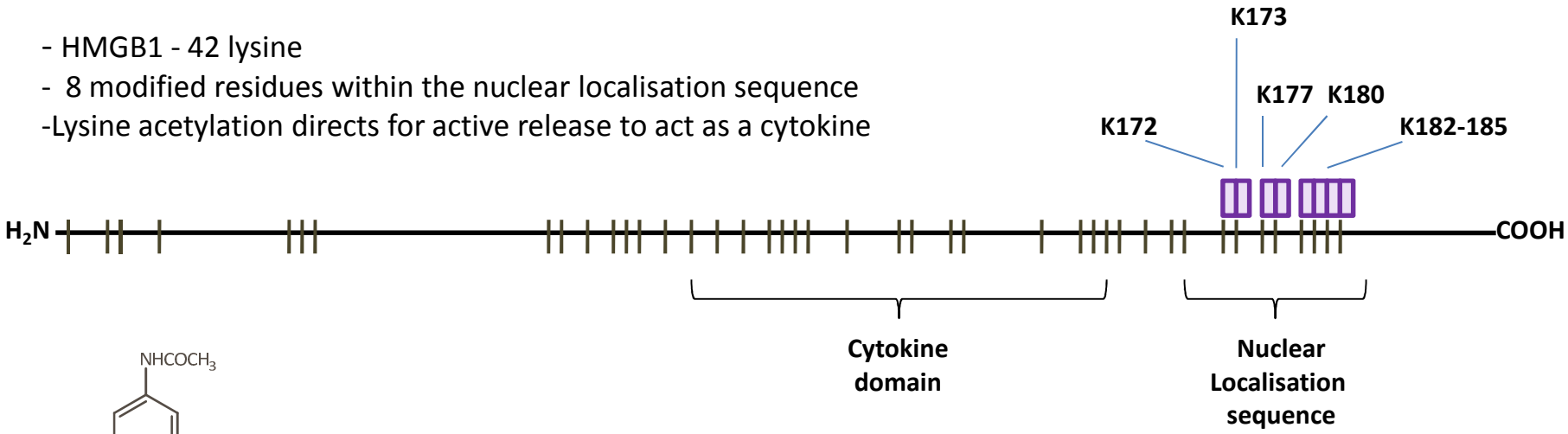
Peter Huebener,[1] Jean-Philippe Pradere,[1] Celine Hernandez,[1] Geum-Youn Gwak,[1] Jorge Matias Caviglia,[1] Xueru Mu,[1] John D. Loike,[2] Rosalind E. Jenkins,[3] Daniel J. Antoine,[3] and Robert F. Schwabe[1]

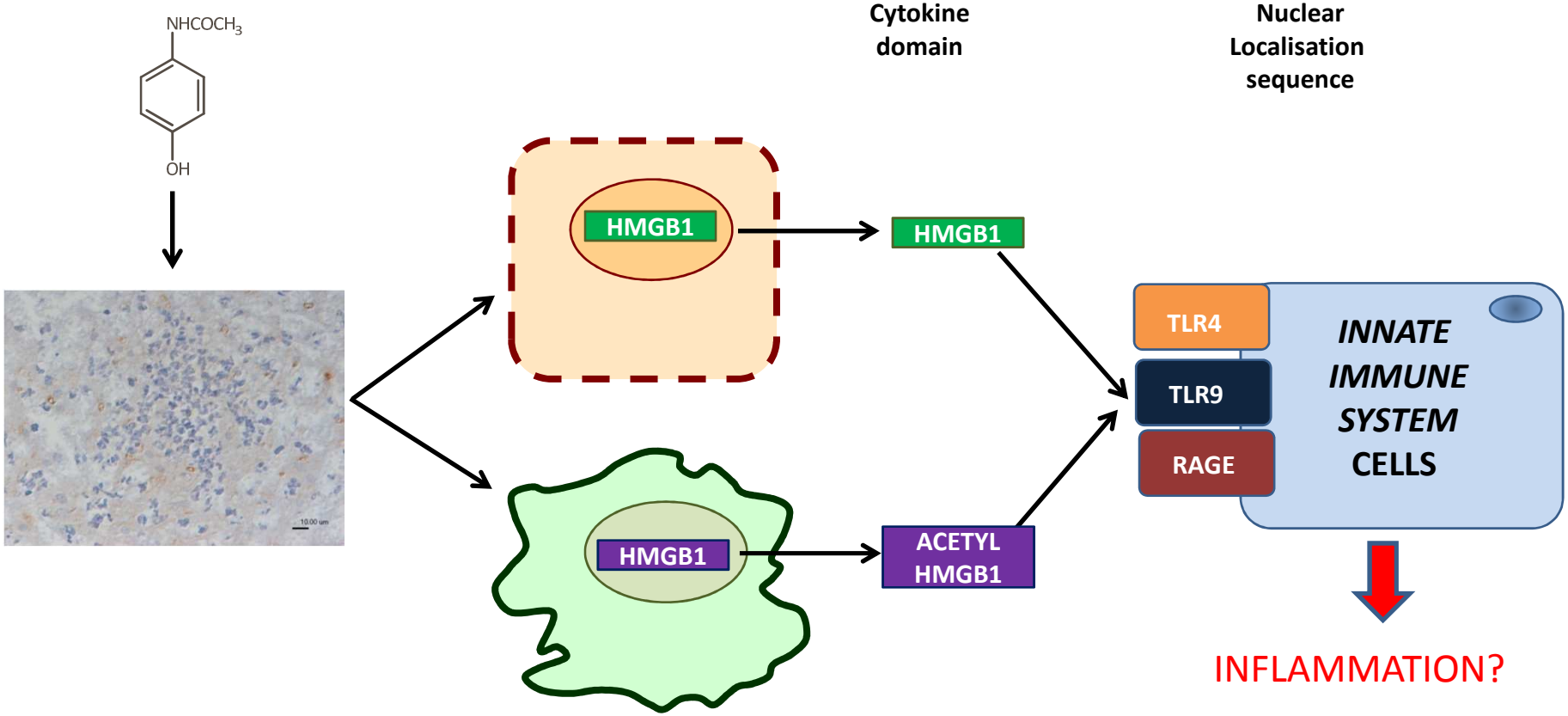
[1]Department of Medicine and [2]Department of Physiology and Cellular Biophysics, Columbia University, New York, New York, USA. [3]MRC Centre for Drug Safety Science, Department of Molecular and Clinical Pharmacology, University of Liverpool, Liverpool, United Kingdom.

- **Mitochondrial DNA and Enzymes (GLDH)**
- **Reflect mitochondrial damage**

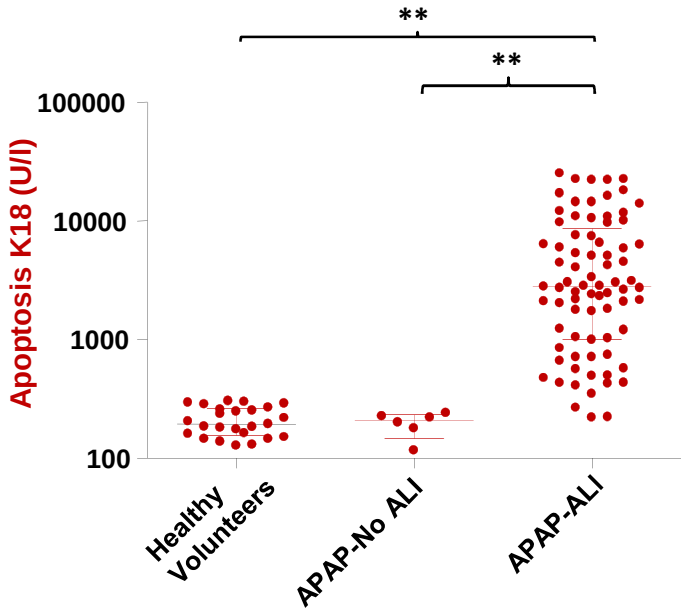
HMGB1 – a biomarker for necrosis and inflammation

- HMGB1 - 42 lysine
- 8 modified residues within the nuclear localisation sequence
-Lysine acetylation directs for active release to act as a cytokine

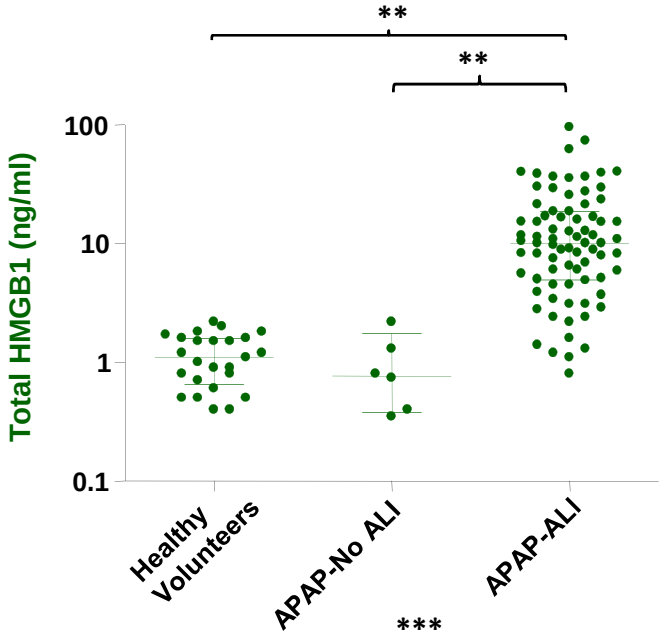




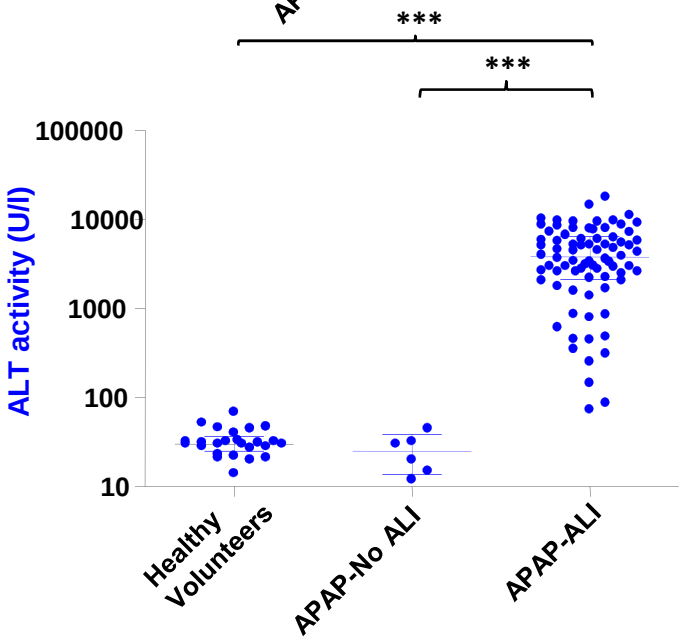
Mechanism of Cell Death - Biomarkers



n = 31, 6, 78



5 – 15% Apoptosis
85 – 95% Necrosis



Molecular forms of HMGB1 and keratin-18 as mechanistic biomarkers for mode of cell death and prognosis during clinical acetaminophen hepatotoxicity

Daniel J. Antoine[1,*], Rosalind E. Jenkins[1], James W. Dear[2], Dominic P. Williams[1], Mitchell R. McGill[3], Matthew R. Sharpe[4], Darren G. Craig[5], Kenneth J. Simpson[5], Hartmut Jaeschke[3], B. Kevin Park[1]

J Hepatology 2012

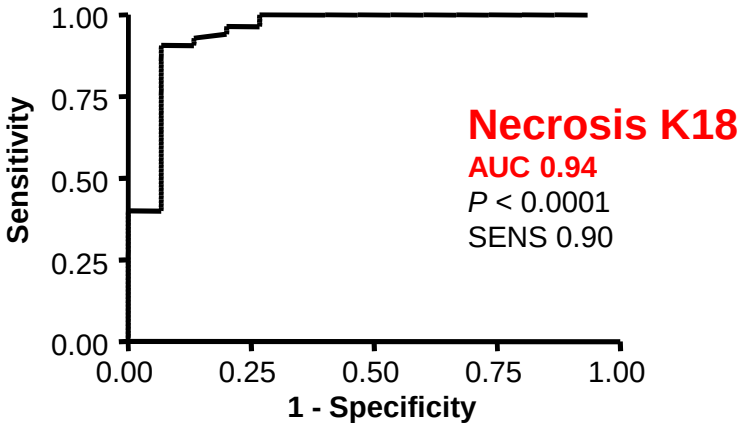
HEPATOLOGY
Official Journal of the American Association for the Study of Liver Diseases

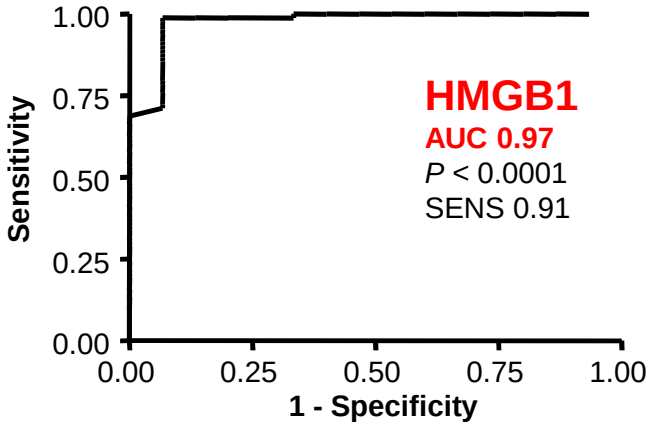
Mechanistic Biomarkers Provide Early and Sensitive Detection of Acetaminophen-Induced Acute Liver Injury at First Presentation to Hospital

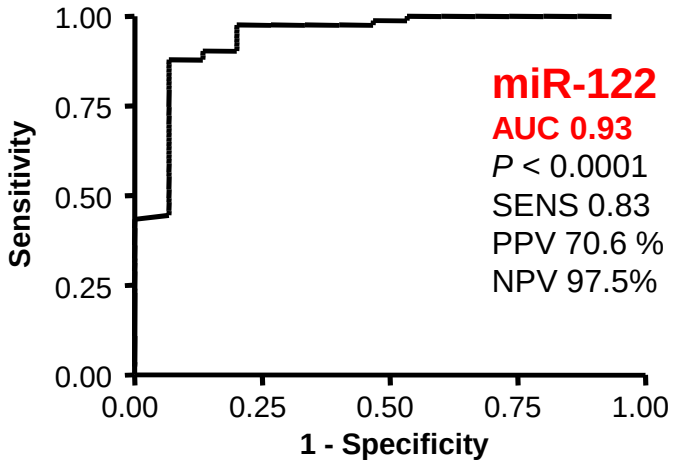
Daniel J. Antoine,[1*] James W. Dear,[2,3*] Philip Starkey Lewis,[1*] Vivien Platt,[1] Judy Coyle,[4] Moyra Masson,[4] Ruben H. Thanacoody,[5] Alasdair J. Gray,[4] David J. Webb,[2,3] Jonathan G. Moggs,[6] D. Nicholas Bateman,[2] Christopher E. Goldring,[1] and B. Kevin Park[1]

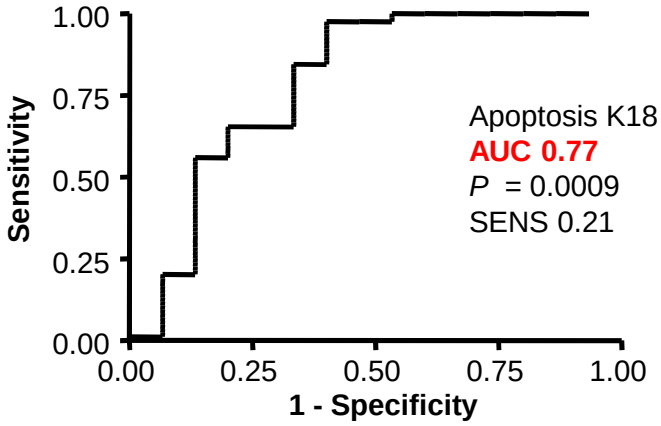
2013

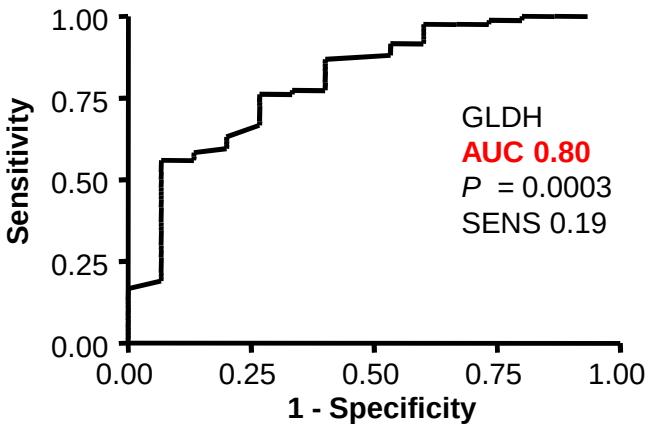
Performance of first presentation biomarkers at predicting acute liver injury (N= 98)









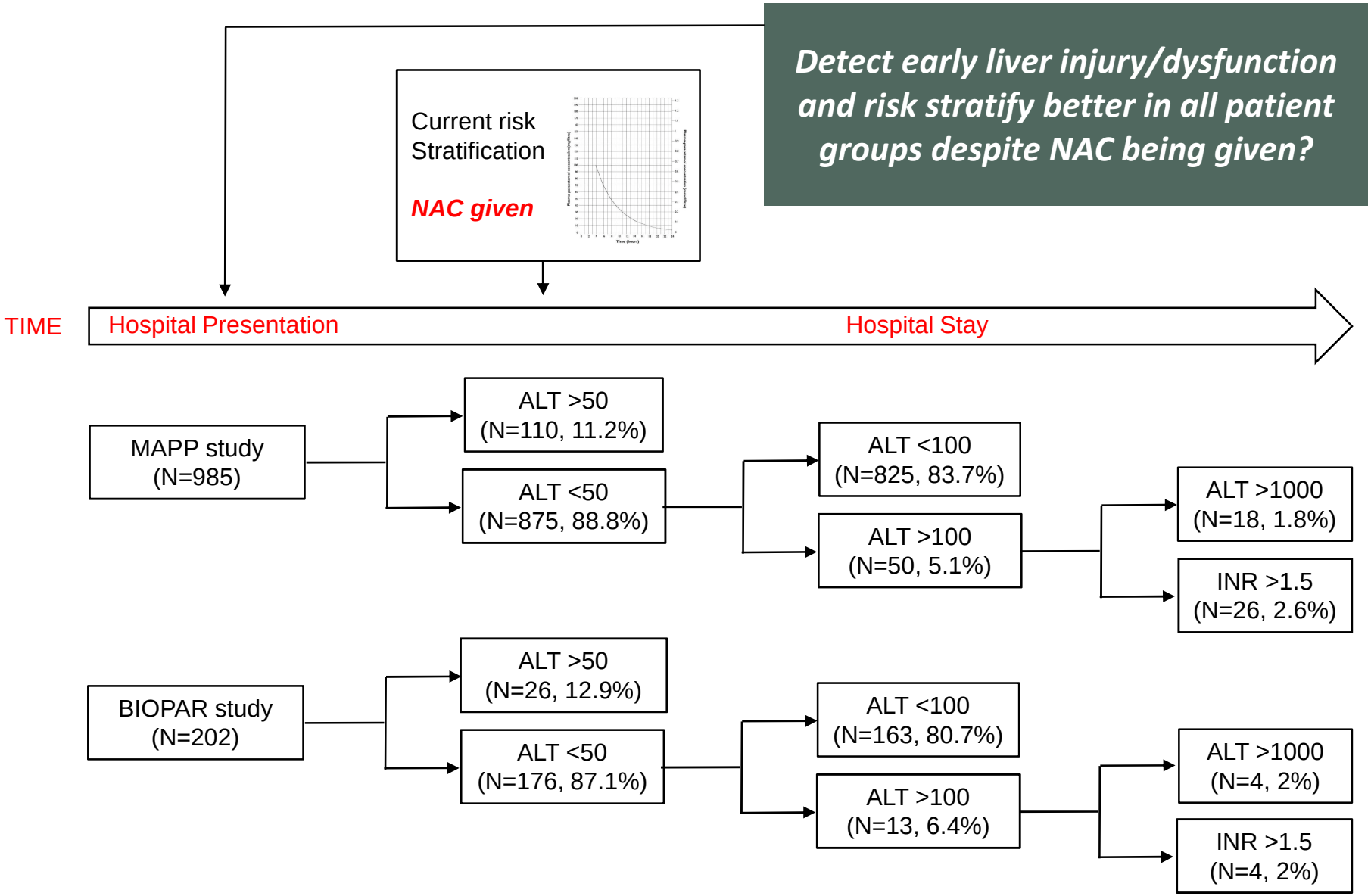


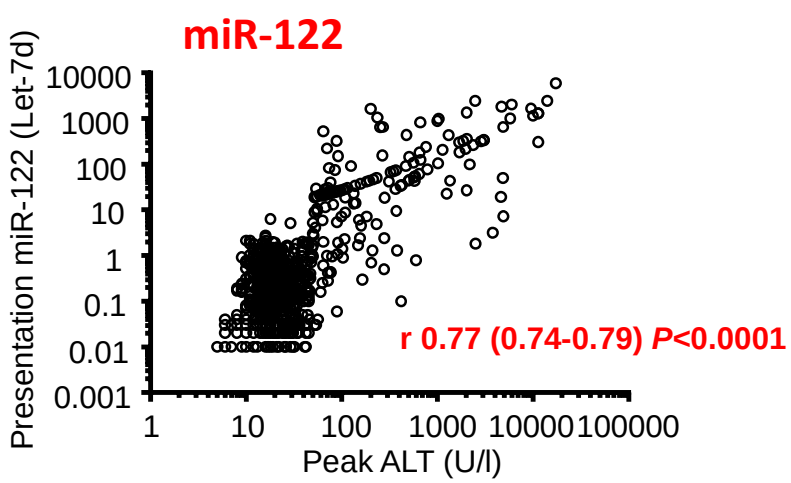
UK wide prospective APAP study – Early risk stratification of liver injury and dysfunction

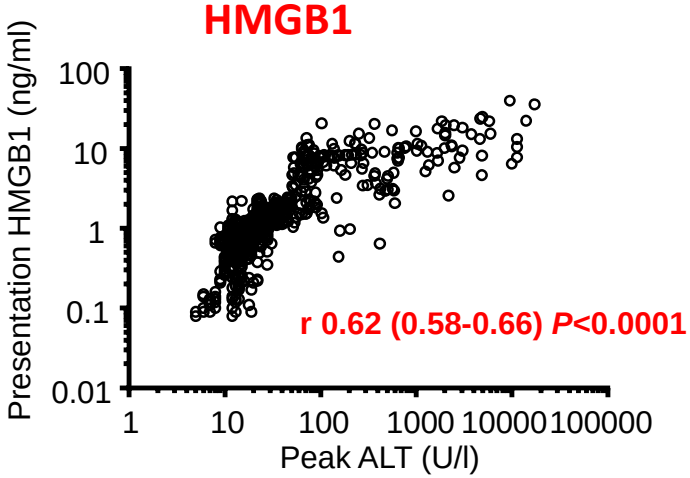
- Mechanistic biomarkers offers earlier detection of ALI following APAP overdose
- Need for prospectively designed studies in man
  - All APAP subgroups
  - Define biomarker performance at front door
  - Risk Stratification
  - Possible entry into novel intervention trials

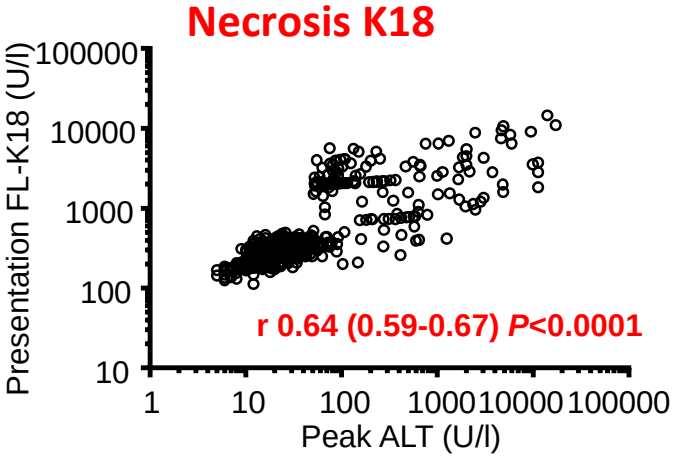
MAPP (8 centres) N = 985
BIOPAR (10 centres) N = 202, 200 HV

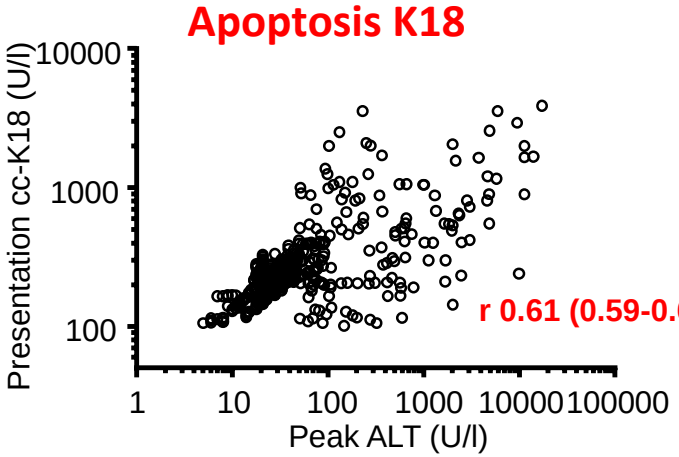
Patient journey – Primary and secondary outcome rates

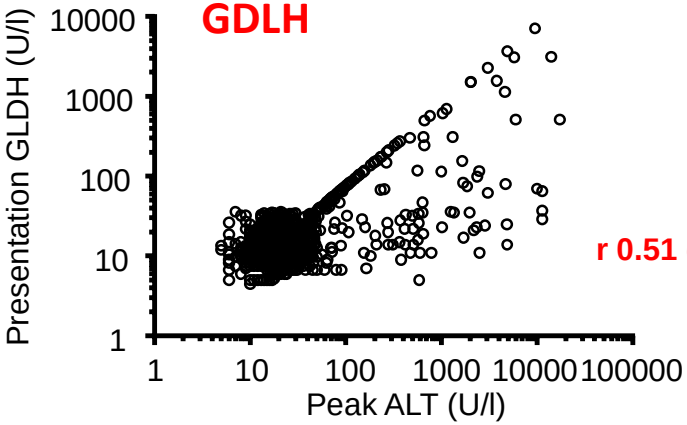






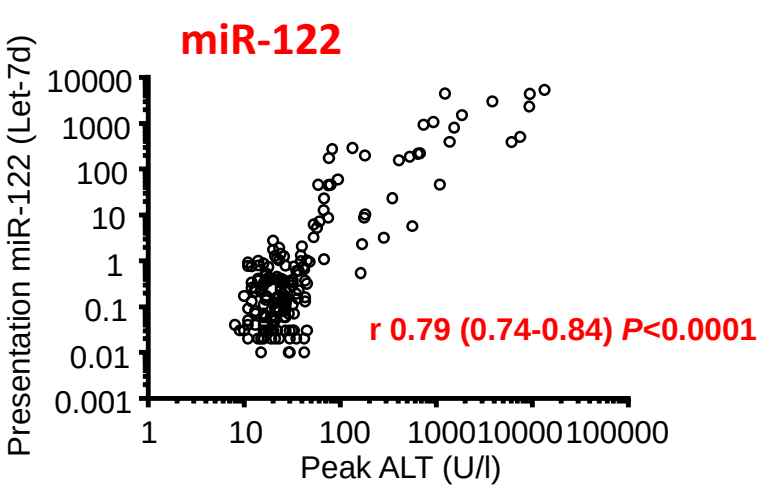


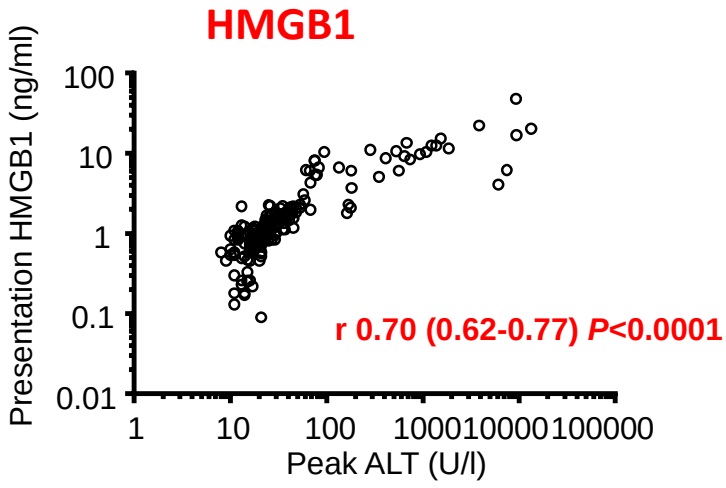


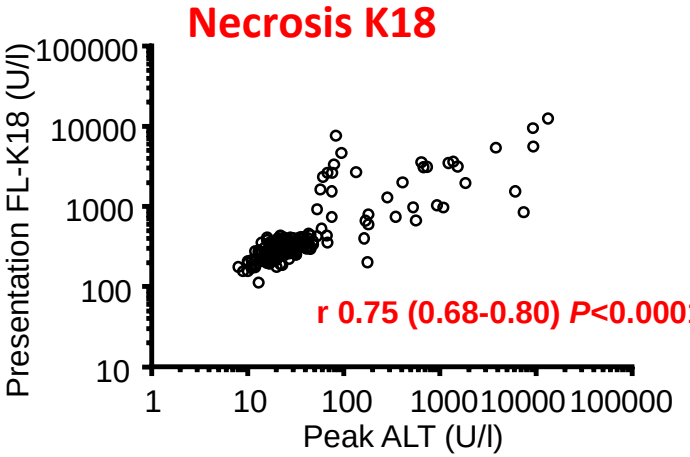


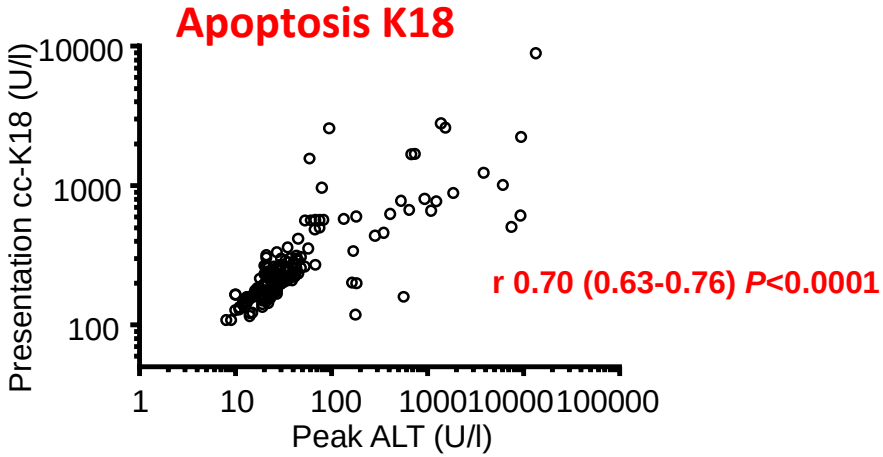
NEW MARKERS CORRELATE WITH PEAK ALT MAPP

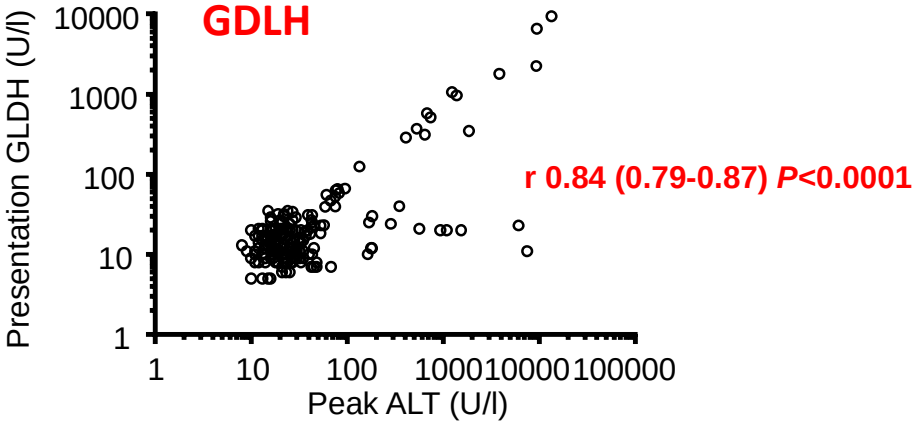
Whole cohort N=985









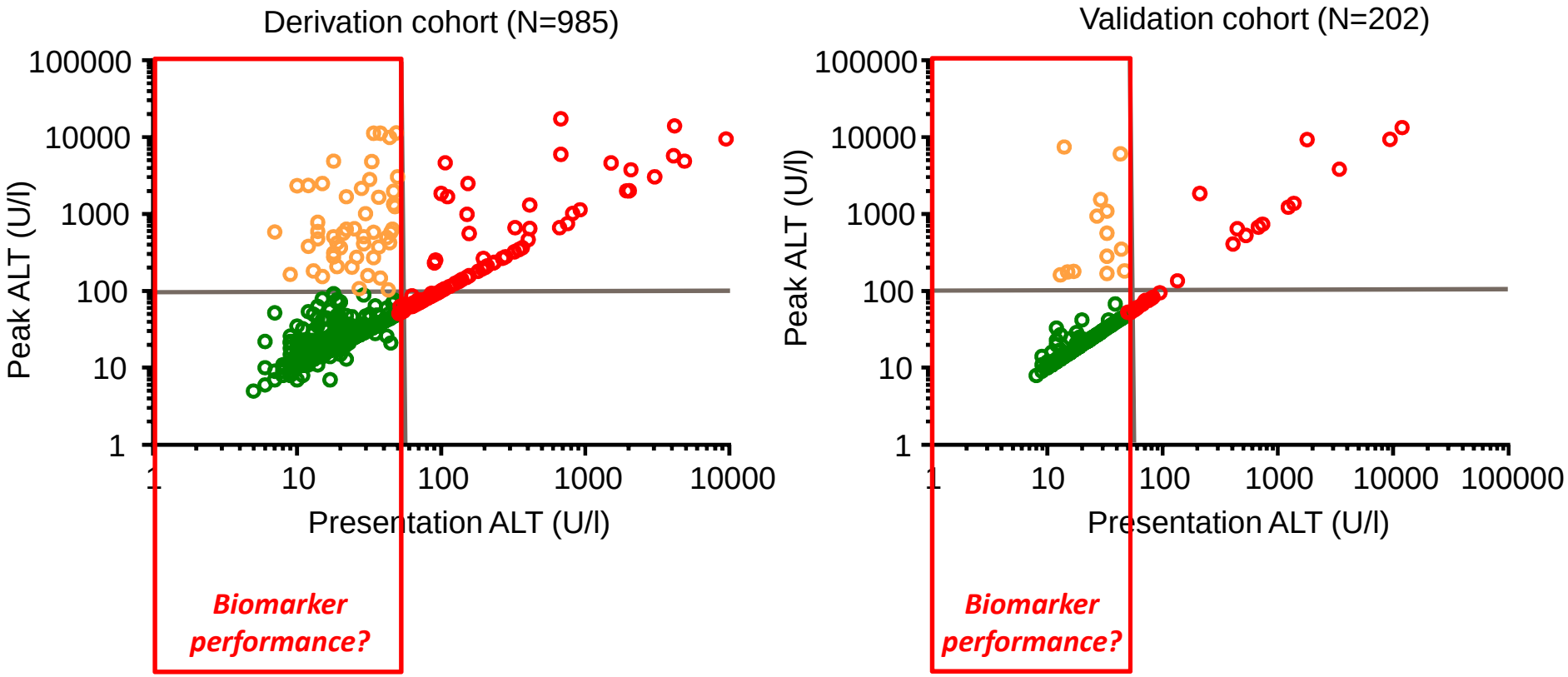


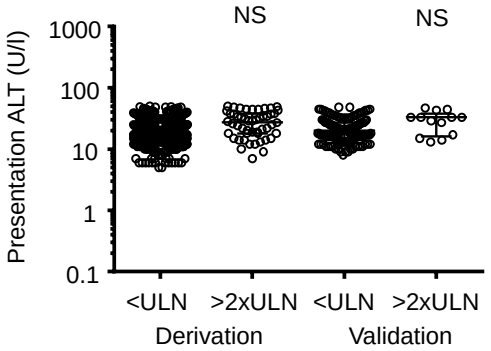
NEW MARKERS CORRELATE WITH PEAK ALT BIOPAR

Whole cohort N=202

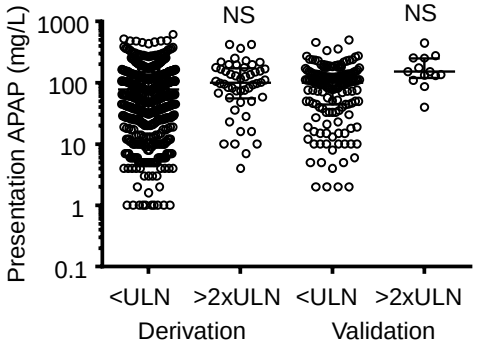
Prospective biomarker study – Results

*Presentation and association with primary outcome – easily spot delayed hepatotoxicity cases **missed** by current tests*



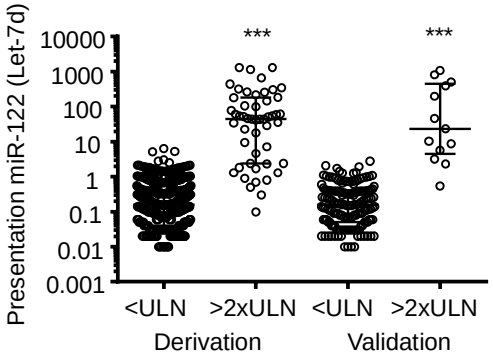


ALT

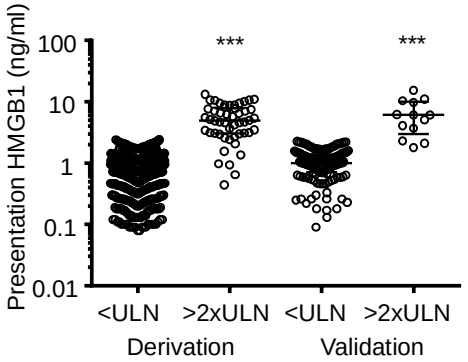


Paracetamol

NORMAL ALT ON PRESENTATION

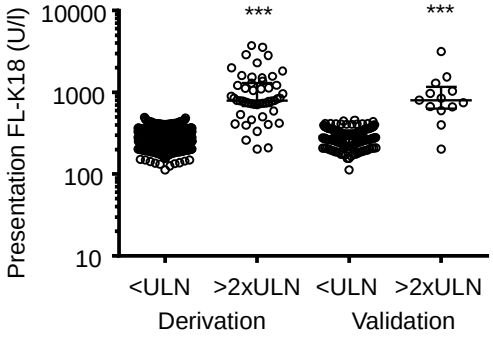


miR-122

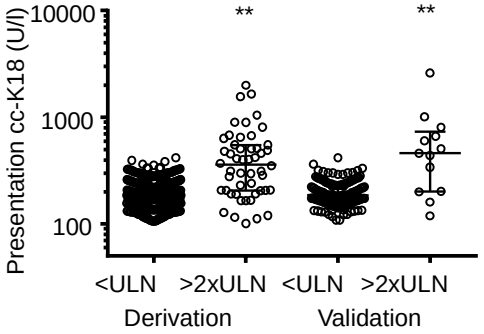


HMGB1

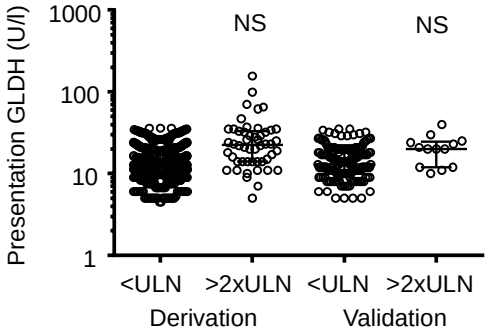
MAPP N=875
BIOPAR N=176



Necrosis K18



Apoptosis K18



GDLH

miR-122, HMGB1 and necrosis K18 are higher in patients who develop ALI

Prediction of reaching primary endpoint in those with normal ALT at presentation

MAPP

Biomarker	ROC-AUC (95% CI)	*P*	Sens at 95% Spec (95% CI)
miR-122	**0.96 (0.93 – 0.99)**	**<0.0001**	**0.84 (0.71 – 0.92)**
HMGB1	**0.94 (0.89 – 0.98)**	**<0.0001**	**0.88 (0.76 – 0.95)**
Necrosis K18	**0.94 (0.89 – 0.99)**	**<0.0001**	**0.88 (0.76 – 0.95)**
Apoptosis K18	0.79 (0.70 – 0.88)	<0.0001	0.60 (0.45 – 0.74)
GLDH	0.74 (0.67 – 0.82)	<0.0001	0.30 (0.18 – 0.44)

BIOPAR

Biomarker	ROC-AUC (95% CI)	*P*	Sens at 95% Spec (95% CI)
HMGB1	**0.98 (0.97 – 1.00)**	**<0.0001**	**0.92 (0.64 – 0.99)**
miR-122	**0.97 (0.94 – 1.00)**	**<0.0001**	**0.92 (0.63 – 0.99)**
Necrosis K18	**0.93 (0.81 – 1.00)**	**<0.0001**	**0.84 (0.54 – 0.98)**
Apoptosis K18	0.80 (0.63 – 0.97)	0.0003	0.69 (0.38 – 0.90)
GLDH	0.65 (0.49 – 0.81)	0.0068	0.09 (0.002 – 0.36)

NORMAL ALT ON PRESENTATION

MAPP N=875
BIOPAR N=176

Multivariable logistic regression analysis – MAPP cohort

Model	ALI Correctly Identified	Non-ALI Correctly Identified
miR-122	44	735
+necrosis.K18	46	811
+apoptosis.K18	47	823
+HMGB1	48	823
Total	**50**	**825**

Only 4 from 875 patients misclassified

NORMAL ALT ON PRESENTATION

Prediction of reaching primary endpoint in staggered overdose

Biomarker	Derivation cohort						Validation cohort					
	ROC-AUC	*P*	Specificity	Sensitivity	PPV	NPV	ROC-AUC	*P*	Specificity	Sensitivity	PPV	NPV
ALT	0.63 (0.48-0.77)	0.1342	0.95	0.38	29.4	95.8	0.57 (0.23-0.91)	0.6203	0.95	0.50	50.0	96.0
APAP concentration	0.57 (0.38-0.77)	0.3451	0.95	0.15	20.0	94.5	0.67 (0.52-0.81)	0.1019	0.95	0.25	25.0	94.0
miR-122	**1.00 (1.00-1.00)**	**<0.0001**	**0.95**	**1.00**	**54.2**	**100.0**	**1.00 (1.00-1.00)**	**<0.0001**	**0.95**	**1.00**	**66.7**	**100.0**
HMGB1	**1.00 (1.00-1.00)**	**<0.0001**	**0.95**	**1.00**	**59.1**	**100.0**	**0.98 (0.94-1.00)**	**<0.0001**	**0.95**	**1.00**	**57.2**	**100.0**
FL-K18	0.99 (0.98-1.00)	<0.0001	0.95	0.92	54.5	99.4	0.76 (0.37-1.00)	0.0800	0.95	0.75	50.0	97.9
cc-K18	0.77 (0.59-0.95)	0.0011	0.95	0.62	44.4	97.3	0.63 (0.22-1.00)	0.3905	0.95	0.50	40.0	95.9
GLDH	0.78 (0.62-0.93)	0.0009	0.95	0.53	36.8	96.8	0.70 (0.47-0.93)	0.1757	0.95	0.25	25.0	94.0

Prediction of INR >1.5 in those with normal ALT and INR at presentation

Biomarker	Derivation cohort						Validation cohort					
	ROC-AUC	*P*	Specificity	Sensitivity	PPV	NPV	ROC-AUC	*P*	Specificity	Sensitivity	PPV	NPV
ALT	0.55 (0.39-0.72)	0.473	0.95	0.23	70.0	52.5	0.57 (0.31-0.80)	0.369	0.95	0.25	0.0	63.6
APAP concentration	0.53 (0.36-0.70)	0.699	0.95	0.00	0.0	45.8	0.55 (0.40-0.79)	0.773	0.95	0.00	0.0	66.7
miR-122	0.73 (0.59-0.88)	0.0043	0.95	0.46	92.3	62.2	0.75 (0.41-1.00)	0.016	0.95	0.50	66.7	80.0
HMGB1	**0.94 (0.88-1.00)**	**<0.0001**	**0.95**	**0.88**	**92.0**	**88.0**	**0.90 (0.73-1.00)**	**0.025**	**0.95**	**0.65**	**75.0**	**88.9**
FL-K18	0.81 (0.69-0.93)	0.0001	0.95	0.27	87.5	54.8	0.86 (0.65-1.00)	0.045	0.95	0.25	50.0	72.3
cc-K18	0.82 (0.70-0.94)	<0.0001	0.95	0.27	77.8	53.7	0.81 (0.55-1.00)	0.008	0.95	0.25	50.0	72.3
GLDH	0.66 (0.51-0.82)	0.042	0.95	0.42	73.3	57.1	0.58 (0.26-0.88)	0.643	0.95	0.25	0.0	66.7

CASE REPORT:
25 year old male
Single overdose of 35g paracetamol at 02:30 (timing supported by Facebook message)
Assessed 4.5h after OD
No risk factors for hepatotoxicity. Paracetamol level 107 mg/L (below nomogram)
Normal biochemical evidence of liver injury
Assessed by senior doctor and not treated
Discharged after psychiatry review

Time from OD (h)	4.5
Paracetamol (mg/L)	107
ALT (U/L) (ULN 50)	34
INR	1.0

British Journal of Clin Pharm 2014;77:904-5.

BJCP British Journal of Clinical Pharmacology

DOI:10.1111/bcp.12214

Letter to the Editors

Early detection of paracetamol toxicity using circulating liver microRNA and markers of cell necrosis

James W. Dear,[1,2] Daniel J. Antoine,[3] Philip Starkey-Lewis,[3] Christopher E. Goldring[3] & B. Kevin Park[3]

[1]National Poisons Information Service Edinburgh, Royal Infirmary of Edinburgh, Edinburgh, [2]University of Edinburgh/British Heart Foundation Centre for Cardiovascular Science, Edinburgh and [3]MRC Centre for Drug Safety Science, Department of Molecular & Clinical Pharmacology, University of Liverpool, Liverpool, UK

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Normal biochemical evidence of liver injury
Assessed by senior doctor and not treated
Discharged after psychiatry review

Represented to hospital 43h after OD
Lethargic and vomiting
Tender abdomen

Time from OD (h)	4.5	43
Paracetamol (mg/L)	107	9
ALT (U/L) (ULN 50)	34	11314
INR	1.0	2.1

British Journal of Clin Pharm 2014;77:904-5.


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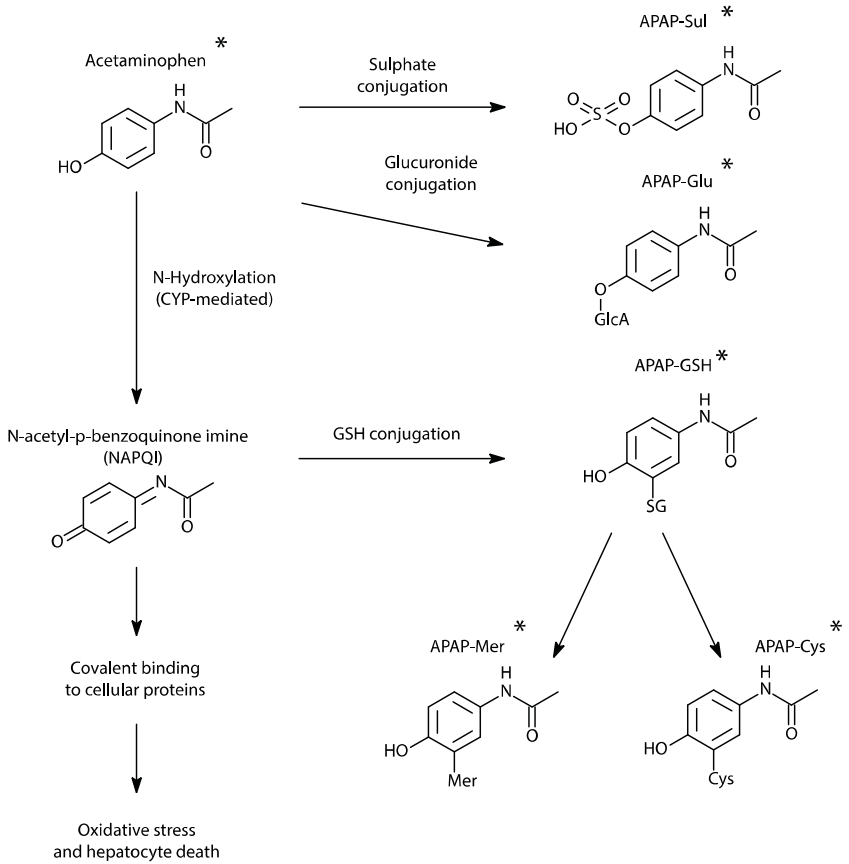
Lethargic and vomiting
Tender abdomen

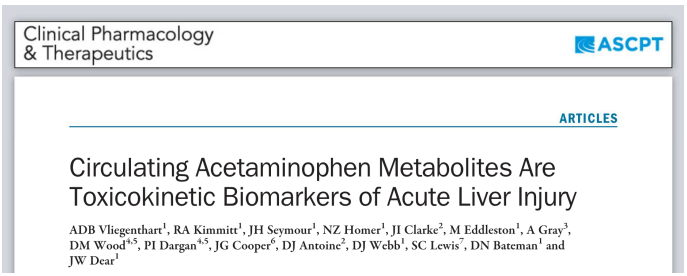
NEW MARKERS CORRECTLY IDENTIFIED
LIFE THREATENING HEPATOTOXICITY MISSED BY CURRENT TESTS

Time from OD (h)	4.5	43
Paracetamol (mg/L)	107	9
ALT (U/L) (ULN 50)	34	11314
INR	1.0	2.1
miR-122 (/ let-7d) (ULN 5.2*)	**261 (x50)**	
HMGB1 (ng/ml) (ULN 0.9*)	**7.2 (x8)**	
Necrosis K18 (U/L) (ULN 480*)	**4018 (x8)**	

*95% prediction interval – no liver injury after overdose
n=82 *Hepatology* 2013

Paracetamol metabolites



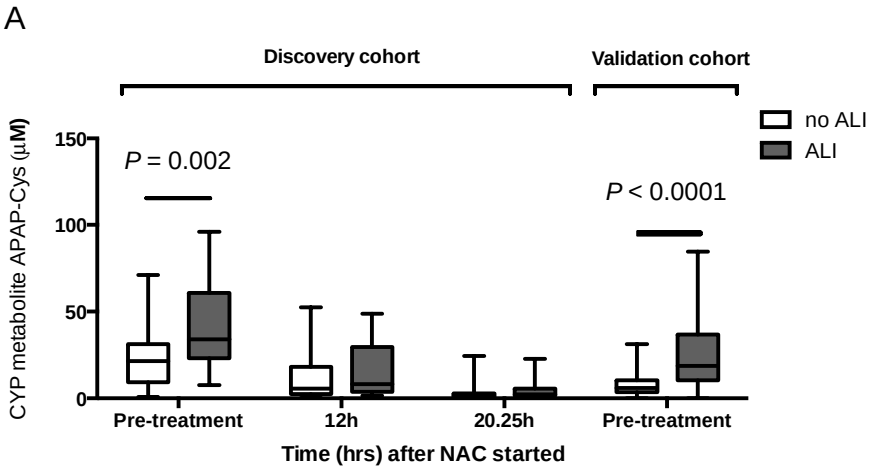

Clinical Pharmacology & Therapeutics

ASCPT

ARTICLES

Circulating Acetaminophen Metabolites Are Toxicokinetic Biomarkers of Acute Liver Injury

ADB Vliegenthart[1], RA Kimmitt[1], JH Seymour[1], NZ Homer[1], JI Clarke[2], M Eddleston[1], A Gray[3], DM Wood[4,5], PI Dargan[4,5], JG Cooper[6], DJ Antoine[2], DJ Webb[1], SC Lewis[7], DN Bateman[1] and JW Dear[1]



	Discovery cohort N=116					Validation cohort N=150				
Metabolite/Biomarker	ROC-AUC (95% CI)	P value	SENS (95% CI)	PPV (%)	NPV (%)	ROC-AUC (95% CI)	P value	SENS (95% CI)	PPV (%)	NPV (%)
APAP-CYS/APAP-Sul	0.91 (0.83-0.98)	**< 0.0001**	0.71 (0.42-0.92)	50	96	0.76 (0.63-0.88)	**0.0003**	0.43 (0.35-0.52)	38	92
CYP%	0.78 (0.67-0.90)	**0.0006**	0.36 (0.13-0.65)	33	91	0.66 (0.51-0.82)	**0.02**	0.11 (0.06-0.17)	14	87
Sum CYP metabolites	0.75 (0.61-0.88)	**0.003**	0.48 (0.38-0.58)	40	93	0.83 (0.71-0.94)	**< 0.0001**	0.44 (0.36-0.53)	39	92
APAP-CYS	0.75 (0.61-0.88)	**0.003**	0.36 (0.13-0.65)	33	91	0.82 (0.71-0.94)	**< 0.0001**	0.44 (0.35-0.52)	39	92
INR	0.70 (0.54-0.86)	**0.03**	0.23 (0.05-0.54)	24	89	0.71 (0.57-0.85)	**0.005**	0.07 (0.03-0.13)	9	87
ALT	0.67 (0.50-0.84)	**0.04**	0.29 (0.08-0.58)	28	90	0.51 (0.35-0.67)	0.86	0.16 (0.03-0.40)	19	88
APAP-Sul	0.65 (0.48-0.82)	0.06	0.50 (0.23-0.77)	41	93	0.53 (0.38-0.67)	0.75	0.11 (0.06-0.17)	14	87
APAP-Glu	0.63 (0.44-0.82)	0.11	0.36 (0.13-0.65)	33	91	0.61 (0.47-0.76)	0.11	0.11 (0.06-0.17)	14	87
APAP-GSH	0.61 (0.46-0.76)	0.19	0.21 (0.05-0.51)	22	89	0.67 (0.61-0.74)	**0.004**	0.41 (0.21-0.64)	37	91
APAP-Mer	0.59 (0.40-0.77)	0.29	0.21 (0.05-0.51)	22	89	0.76 (0.62-0.90)	**0.0003**	0.26 (0.19-0.34)	27	89
APAP LC/MS	0.50 (0.33-0.67)	0.97	0.14 (0.02-0.43)	16	88	0.57 (0.41-0.73)	0.32	0.05 (0.02-0.11)	7	87
APAP hospital lab	0.55 (0.37-0.73)	0.78	0.00 (0.00-0.04)	0	87	0.58 (0.42-0.73)	0.29	0.09 (0.04-0.15)	12	87

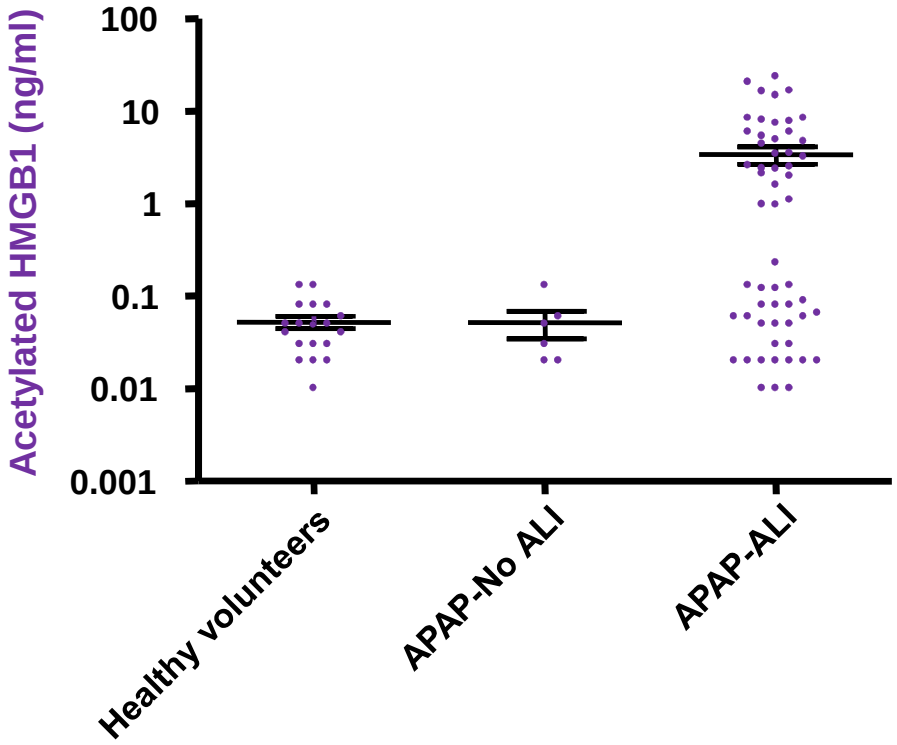
PARACETAMOL QUESTION 2:

Who has a serious ALT rise?

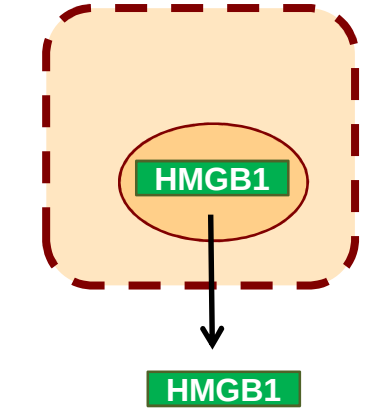
- Improved prognostic stratification

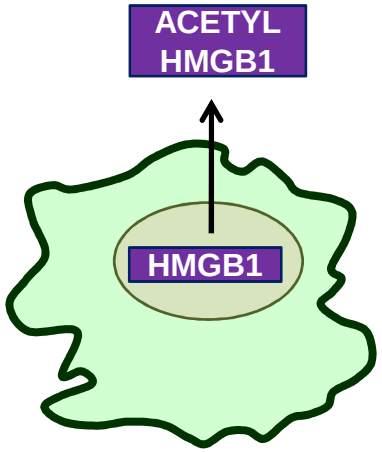
Acetylated HMGB1 – biomarker of immune cell activation

- Clinical APAP overdose



All patients



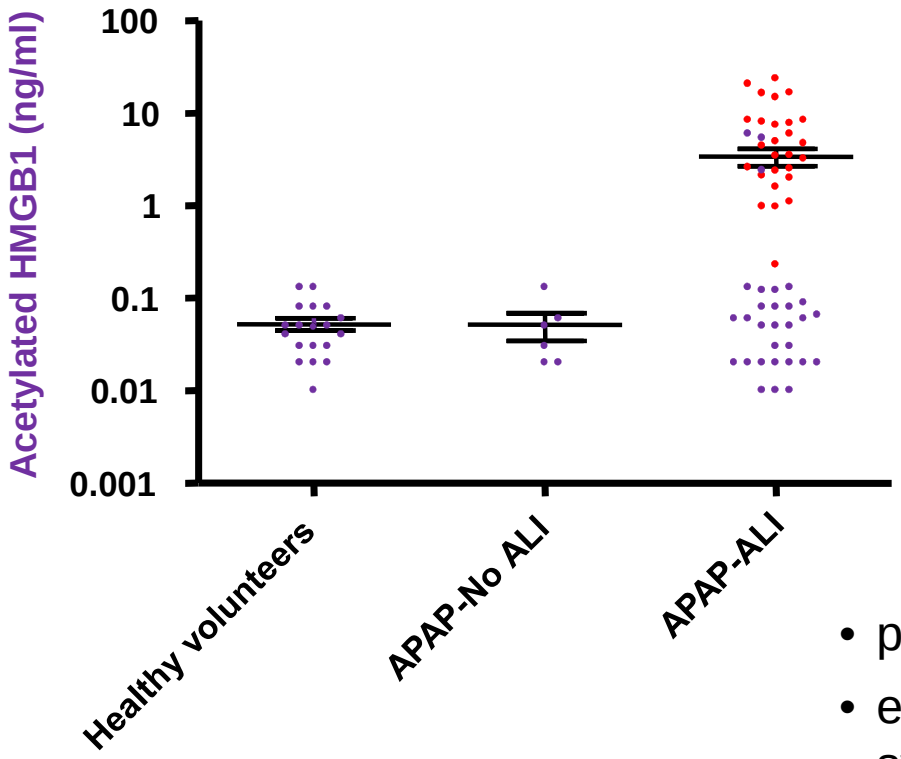


Acetylated HMGB1 – outcome prediction

- Clinical APAP overdose

Molecular forms of HMGB1 and keratin-18 as mechanistic biomarkers for mode of cell death and prognosis during clinical acetaminophen hepatotoxicity

Daniel J. Antoine[1,*], Rosalind E. Jenkins[1], James W. Dear[2], Dominic P. Williams[1], Mitchell R. McGill[3], Matthew R. Sharpe[4], Darren G. Craig[5], Kenneth J. Simpson[5], Hartmut Jaeschke[3], B. Kevin Park[1]



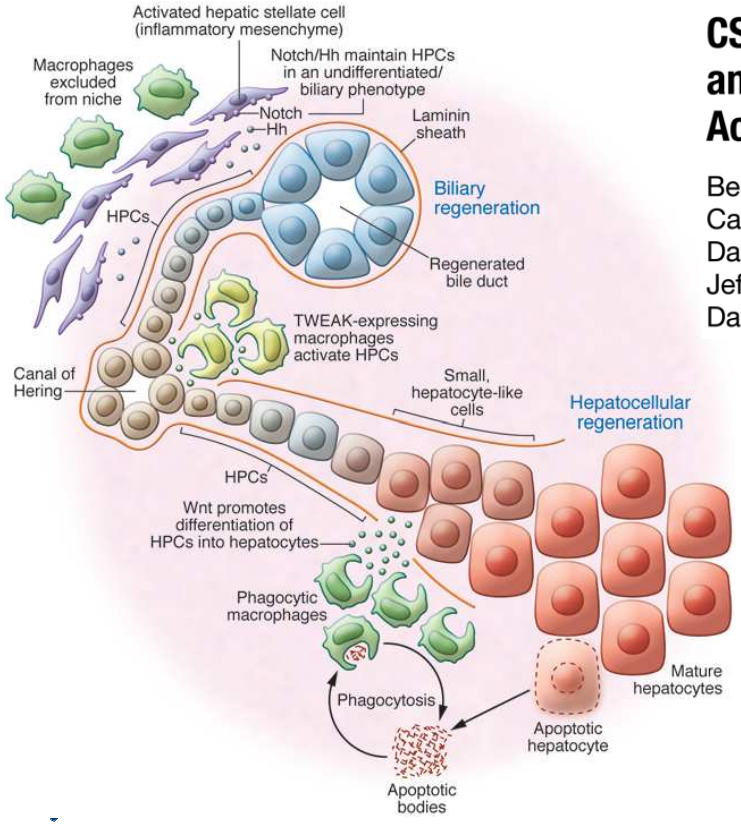
Died / Required Liver transplant

Spontaneous survivors

- potential novel biomarker
- evidence in man for innate immune system
- Multi-cellular event

Biomarkers of hepatic regeneration & survival

- CSF-1
- Promotes Macrophage recruitment and hepatic regeneration



BASIC AND TRANSLATIONAL—LIVER

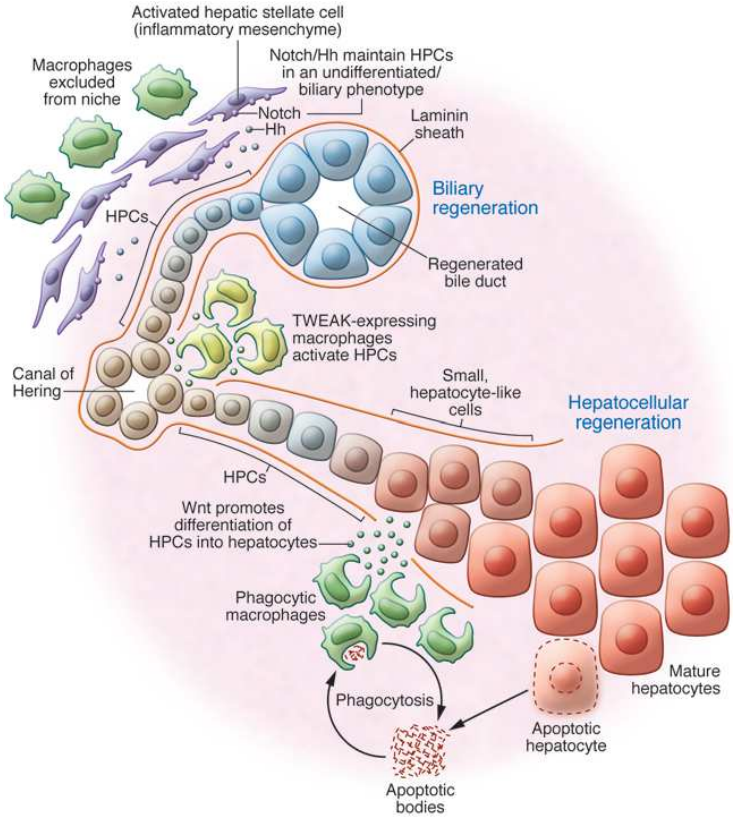
CSF1 Restores Innate Immunity After Liver Injury in Mice and Serum Levels Indicate Outcomes of Patients With Acute Liver Failure

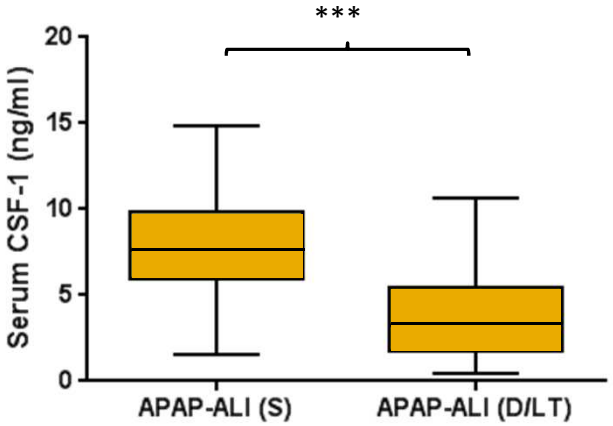
Benjamin M. Stutchfield,[1,2] Daniel J. Antoine,[3] Alison C. Mackinnon,[1] Deborah J. Gow,[4] Calum C. Bain,[5] Catherine A. Hawley,[6] Michael J. Hughes,[2] Benjamin Francis,[7] Davina Wojtacha,[1] Tak Y. Man,[1] James W. Dear,[8] Luke R. Devey,[6] Alan M. Mowat,[5] Jeffrey W. Pollard,[9] B. Kevin Park,[3] Stephen J. Jenkins,[6] Kenneth J. Simpson,[2] David A. Hume,[3] Stephen J. Wigmore,[2] and Stuart J. Forbes[1]

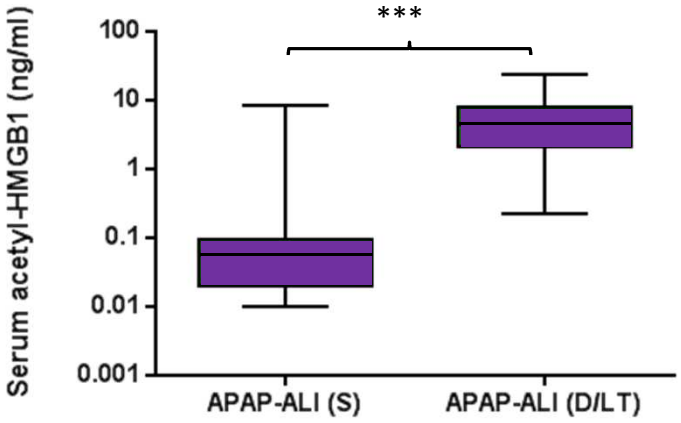
Gastroenterology 2015

Biomarkers of hepatic regeneration & survival

- CSF-1
- Promotes Macrophage recruitment and hepatic regeneration
- **APAP overdose**







Acute kidney injury is a key predictor of poor outcome with DILI

Eur J Clin Pharmacol (2009) 65:163–168
DOI 10.1007/s00228-008-0580-9

PHARMACODYNAMICS

Renal injury at first presentation as a predictor for poor outcome in severe paracetamol poisoning referred to a liver transplant unit

N. Pakravan · K. J. Simpson · W. S. Waring · C. M. Bates · D. N. Bateman

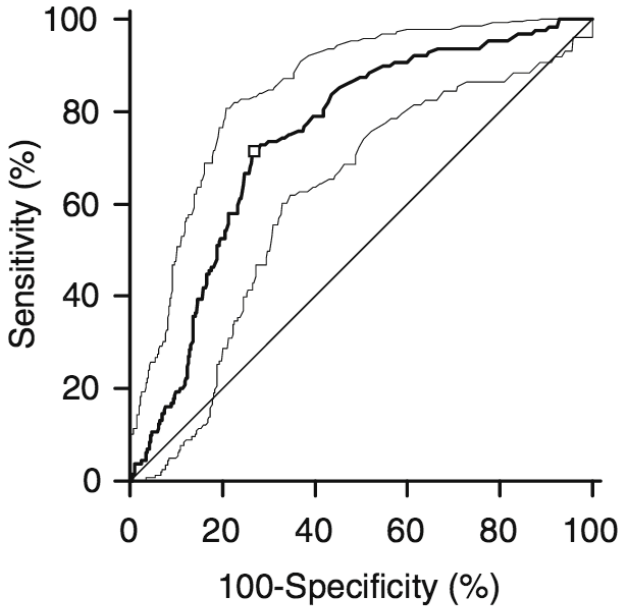
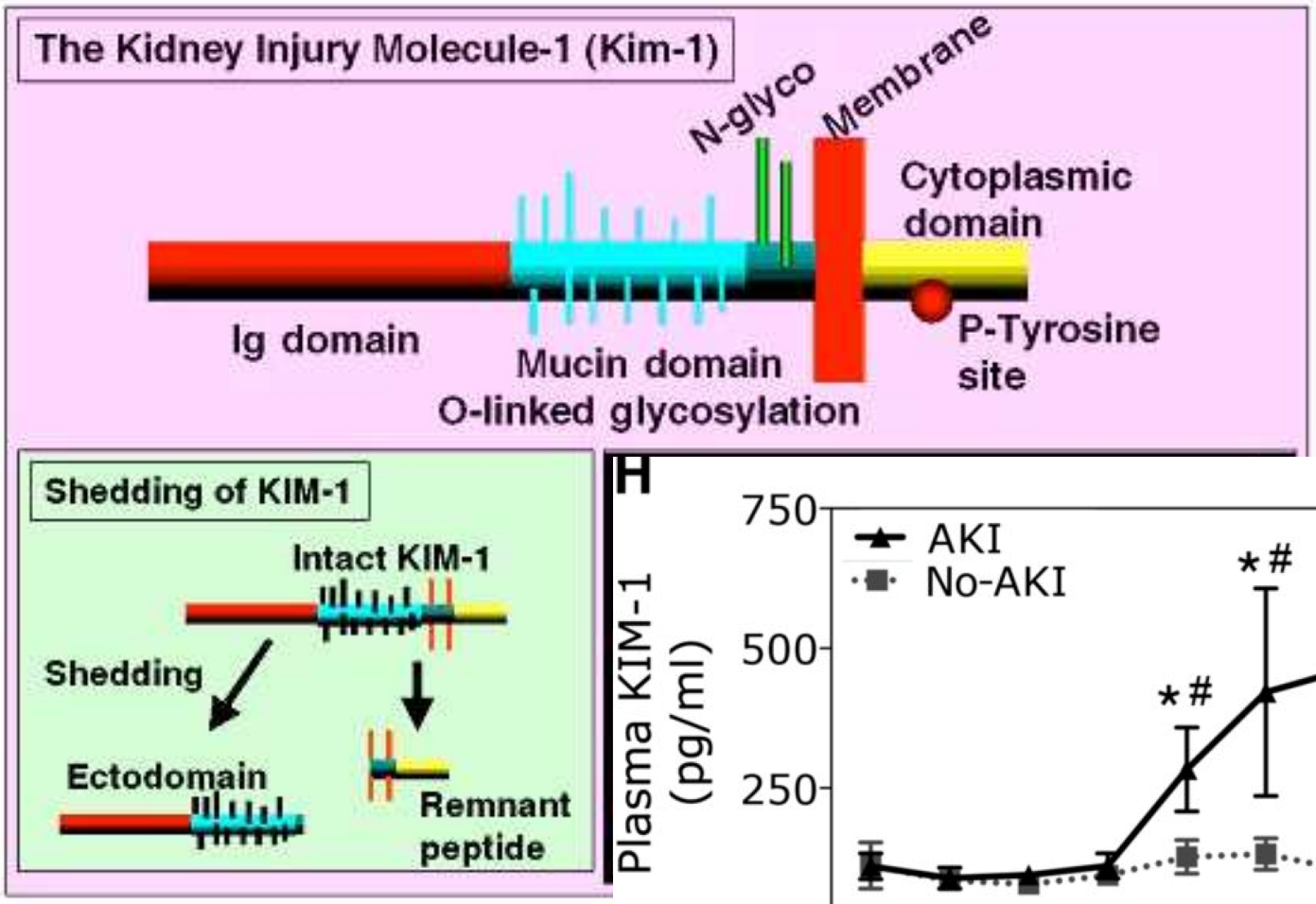


Fig. 1 The receiver operating characteristic (ROC) curve for referral creatinine versus "King's College poor outcome" area under the ROC curve (AUC)=74.3% [95% confidence interval (CI) 70.1–78.1%, significance level p=0.0001 (versus 0.5 line by z test)]. The most "accurate' predictor" is referral creatinine >123 µmol/l, sensitivity= 71.3% (95% CI 62.7–78.9%) and specificity=73.3% (95% CI 68.3–77.9%)

Problems with creatinine

1. **Slow to report AKI**
2. **Non specific**
3. **No mechanistic information**



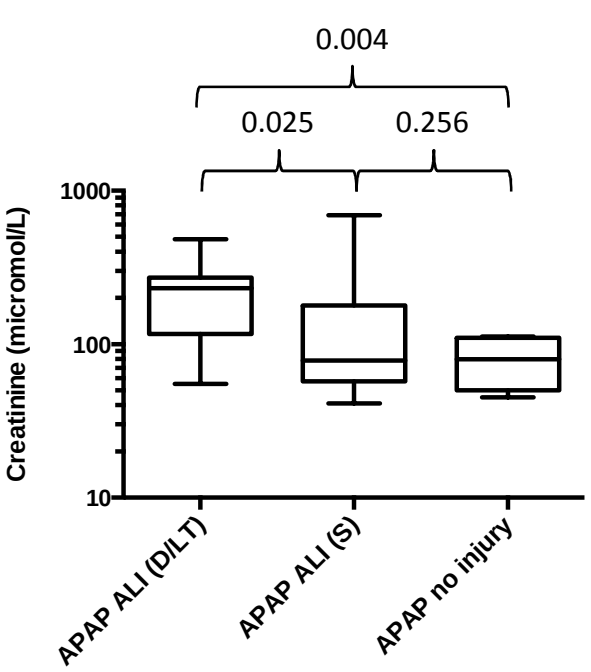
BRIEF COMMUNICATION www.jasn.org

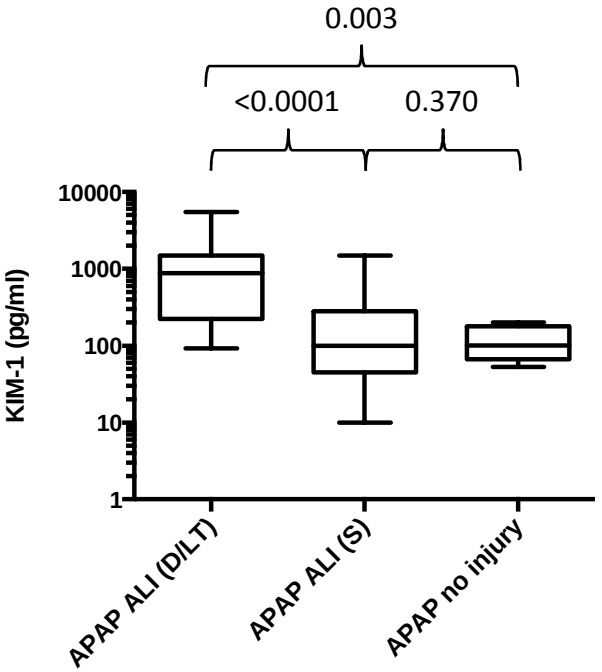
Blood Kidney Injury Molecule-1 Is a Biomarker of Acute and Chronic Kidney Injury and Predicts Progression to ESRD in Type I Diabetes

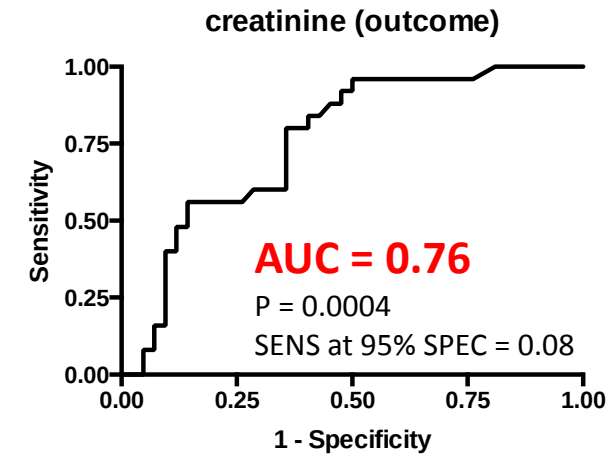
Venkata S. Sabbisetti,* Sushrut S. Waikar,* Daniel J. Antoine,† Adam Smiles,‡ Chang Wang,* Abinaya Ravisankar,* Kazumi Ito,* Sahil Sharma,* Swetha Ramadesikan,* Michelle Lee,§ Rebeccah Briskin,§ Philip L. De Jager,§ Thanh Thu Ngo,* Mark Radlinski,* James W. Dear,‖ Kevin B. Park,† Rebecca Betensky,¶ Andrzej S. Krolewski,‡ and Joseph V. Bonventre*

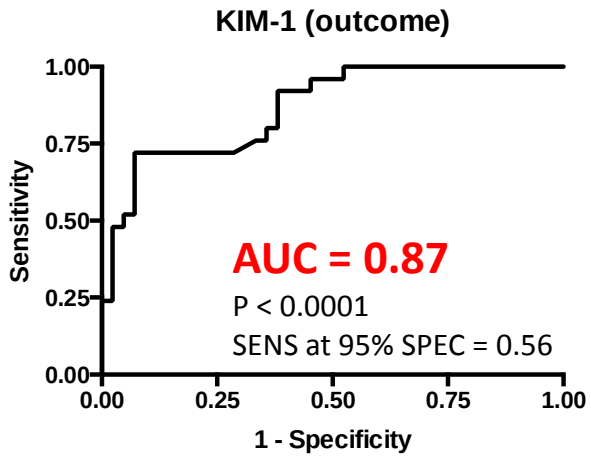
J Am Soc Nephrol. 2014;25:2177-86

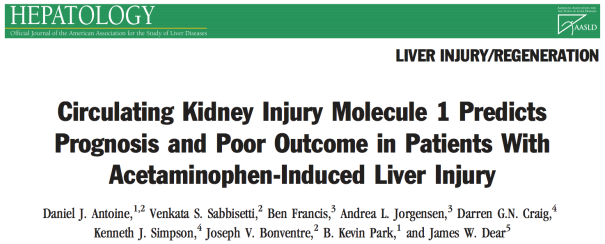
Plasma KIM-1 outperforms serum creatinine in the prognostic assessment of patient outcome following paracetamol overdose










HEPATOLOGY
Official Journal of the American Association for the Study of Liver Diseases
AASLD

LIVER INJURY/REGENERATION

Circulating Kidney Injury Molecule 1 Predicts Prognosis and Poor Outcome in Patients With Acetaminophen-Induced Liver Injury

Daniel J. Antoine,[1,2] Venkata S. Sabbisetti,[2] Ben Francis,[3] Andrea L. Jorgensen,[3] Darren G.N. Craig,[4] Kenneth J. Simpson,[4] Joseph V. Bonventre,[2] B. Kevin Park,[1] and James W. Dear[5]

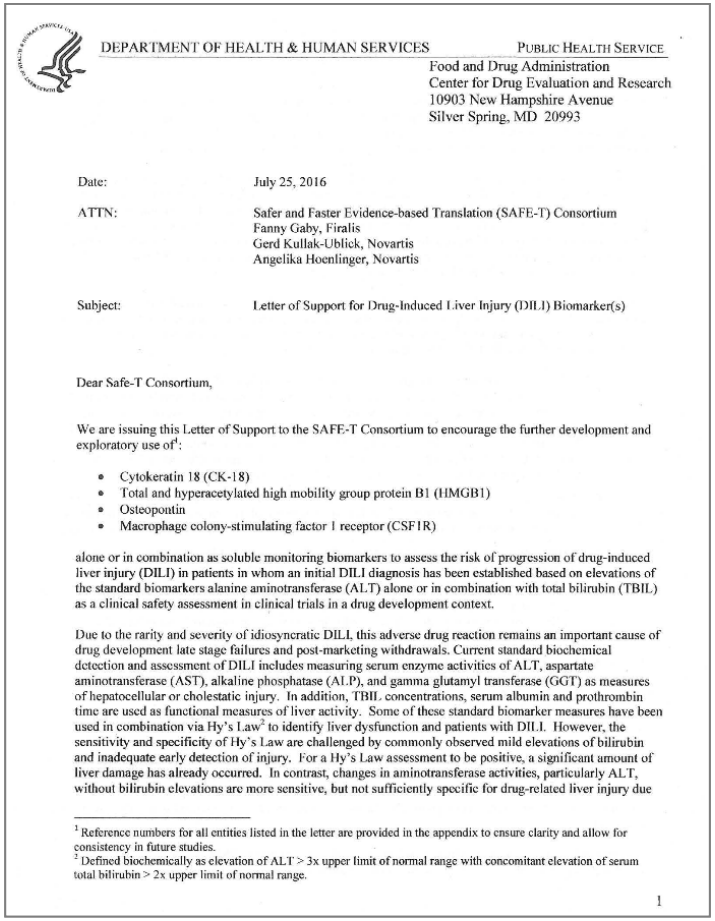
N=74

Hepatology 2015;62:591-9

Regulatory endorsement for further qualification

SAFE-T

DILIN
Drug-Induced Liver Injury Network



DEPARTMENT OF HEALTH & HUMAN SERVICES — PUBLIC HEALTH SERVICE

Food and Drug Administration
Center for Drug Evaluation and Research
10903 New Hampshire Avenue
Silver Spring, MD 20993

Date: July 25, 2016

ATTN: Safer and Faster Evidence-based Translation (SAFE-T) Consortium
Fanny Gaby, Firalis
Gerd Kullak-Ublick, Novartis
Angelika Hoenlinger, Novartis

Subject: Letter of Support for Drug-Induced Liver Injury (DILI) Biomarker(s)

Dear Safe-T Consortium,

We are issuing this Letter of Support to the SAFE-T Consortium to encourage the further development and exploratory use of[1]:

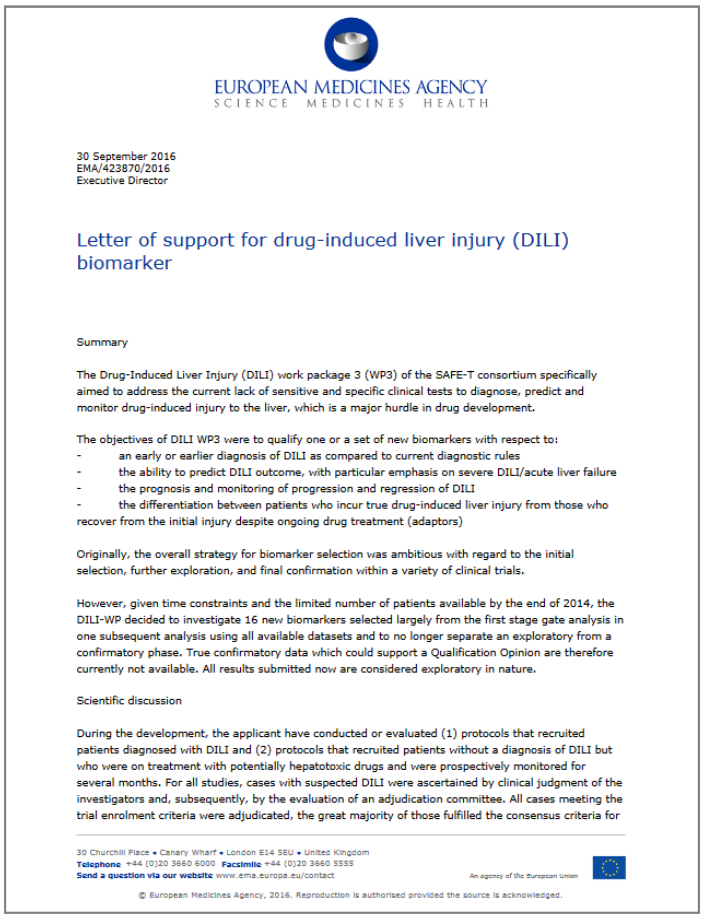
- Cytokeratin 18 (CK-18)
- Total and hyperacetylated high mobility group protein B1 (HMGB1)
- Osteopontin
- Macrophage colony-stimulating factor 1 receptor (CSF1R)

alone or in combination as soluble monitoring biomarkers to assess the risk of progression of drug-induced liver injury (DILI) in patients in whom an initial DILI diagnosis has been established based on elevations of the standard biomarkers alanine aminotransferase (ALT) alone or in combination with total bilirubin (TBIL) as a clinical safety assessment in clinical trials in a drug development context.

Due to the rarity and severity of idiosyncratic DILI, this adverse drug reaction remains an important cause of drug development late stage failures and post-marketing withdrawals. Current standard biochemical detection and assessment of DILI includes measuring serum enzyme activities of ALT, aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma glutamyl transferase (GGT) as measures of hepatocellular or cholestatic injury. In addition, TBIL concentrations, serum albumin and prothrombin time are used as functional measures of liver activity. Some of these standard biomarker measures have been used in combination via Hy's Law[2] to identify liver dysfunction and patients with DILI. However, the sensitivity and specificity of Hy's Law are challenged by commonly observed mild elevations of bilirubin and inadequate early detection of injury. For a Hy's Law assessment to be positive, a significant amount of liver damage has already occurred. In contrast, changes in aminotransferase activities, particularly ALT, without bilirubin elevations are more sensitive, but not sufficiently specific for drug-related liver injury due

[1] Reference numbers for all entities listed in the letter are provided in the appendix to ensure clarity and allow for consistency in future studies.
[2] Defined biochemically as elevation of ALT > 3x upper limit of normal range with concomitant elevation of serum total bilirubin > 2x upper limit of normal range.

1



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

30 September 2016
EMA/423870/2016
Executive Director

Letter of support for drug-induced liver injury (DILI) biomarker

Summary

The Drug-Induced Liver Injury (DILI) work package 3 (WP3) of the SAFE-T consortium specifically aimed to address the current lack of sensitive and specific clinical tests to diagnose, predict and monitor drug-induced injury to the liver, which is a major hurdle in drug development.

The objectives of DILI WP3 were to qualify one or a set of new biomarkers with respect to:
- an early or earlier diagnosis of DILI as compared to current diagnostic rules
- the ability to predict DILI outcome, with particular emphasis on severe DILI/acute liver failure
- the prognosis and monitoring of progression and regression of DILI
- the differentiation between patients who incur true drug-induced liver injury from those who recover from the initial injury despite ongoing drug treatment (adaptors)

Originally, the overall strategy for biomarker selection was ambitious with regard to the initial selection, further exploration, and final confirmation within a variety of clinical trials.

However, given time constraints and the limited number of patients available by the end of 2014, the DILI-WP decided to investigate 16 new biomarkers selected largely from the first stage gate analysis in one subsequent analysis using all available datasets and to no longer separate an exploratory from a confirmatory phase. True confirmatory data which could support a Qualification Opinion are therefore currently not available. All results submitted now are considered exploratory in nature.

Scientific discussion

During the development, the applicant have conducted or evaluated (1) protocols that recruited patients diagnosed with DILI and (2) protocols that recruited patients without a diagnosis of DILI but who were on treatment with potentially hepatotoxic drugs and were prospectively monitored for several months. For all studies, cases with suspected DILI were ascertained by clinical judgment of the investigators and, subsequently, by the evaluation of an adjudication committee. All cases meeting the trial enrolment criteria were adjudicated, the great majority of those fulfilled the consensus criteria for

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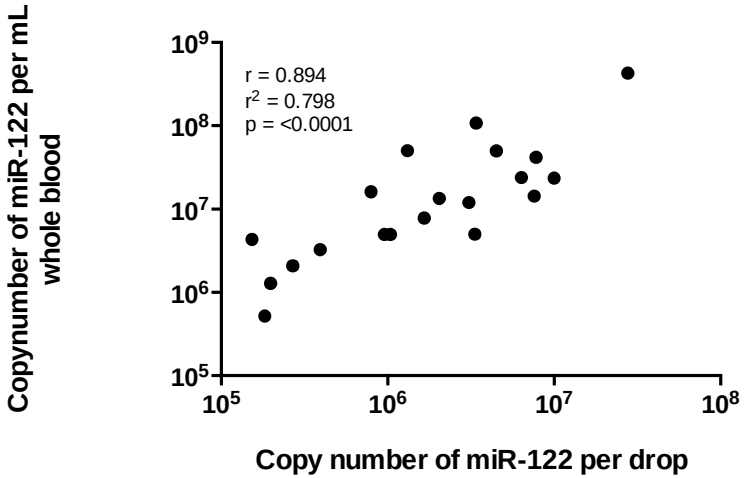
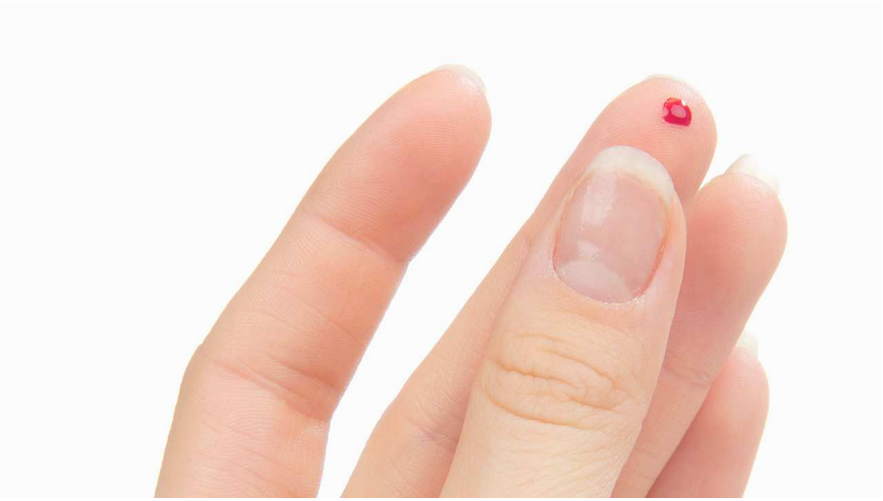
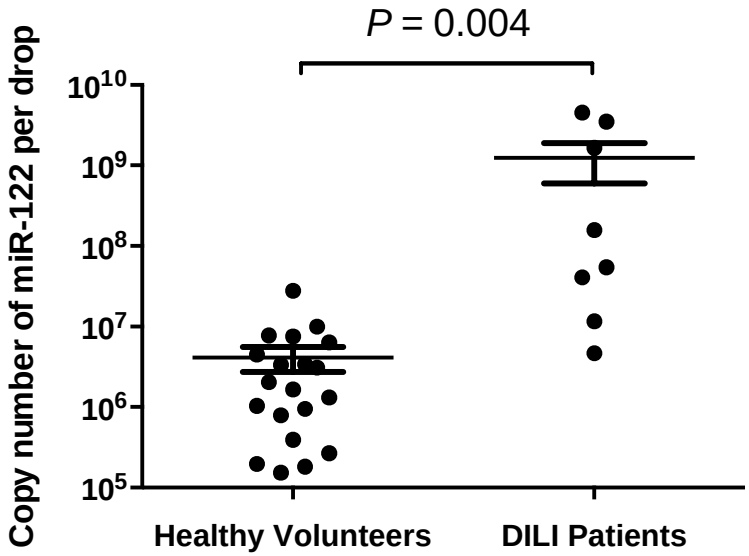
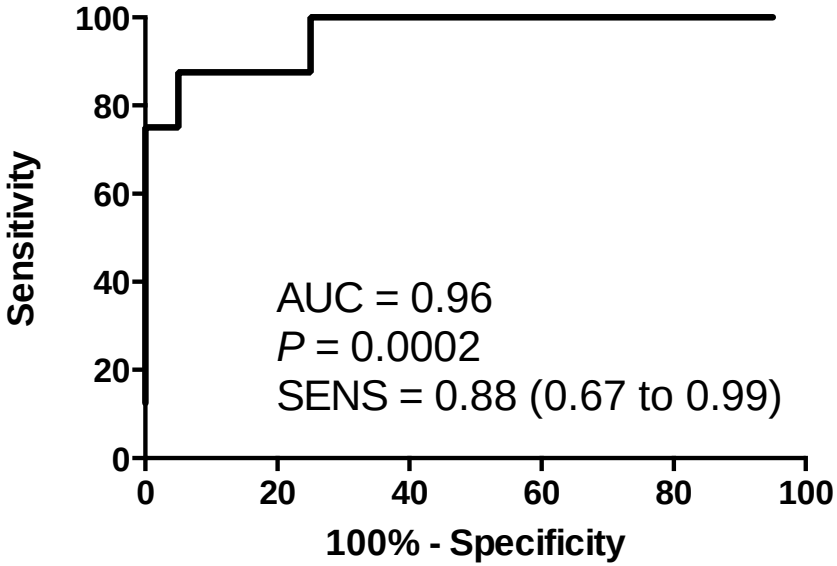
Translation to point of care

Biomarker can be measured in a blood drop from a standard clinical finger prick



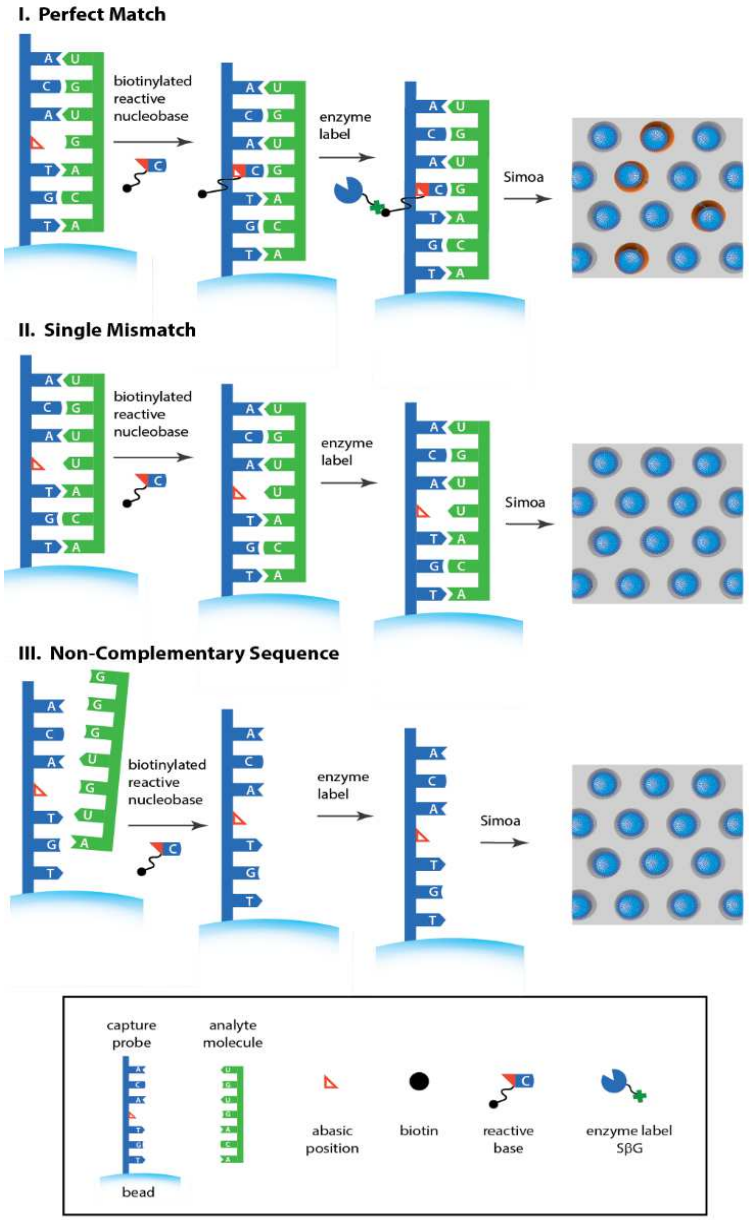


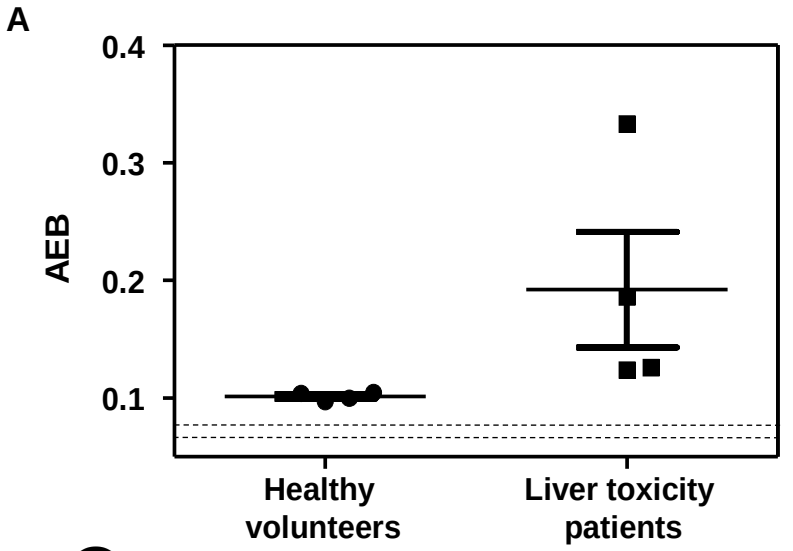


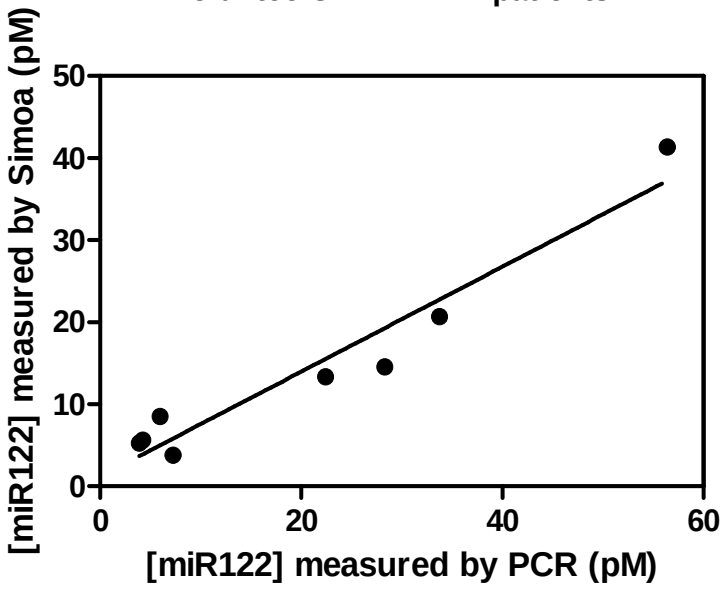


Vliegenthart et al BJCP 2017 – published online

Destina genomics – a rapid sensitive PCR free detection system

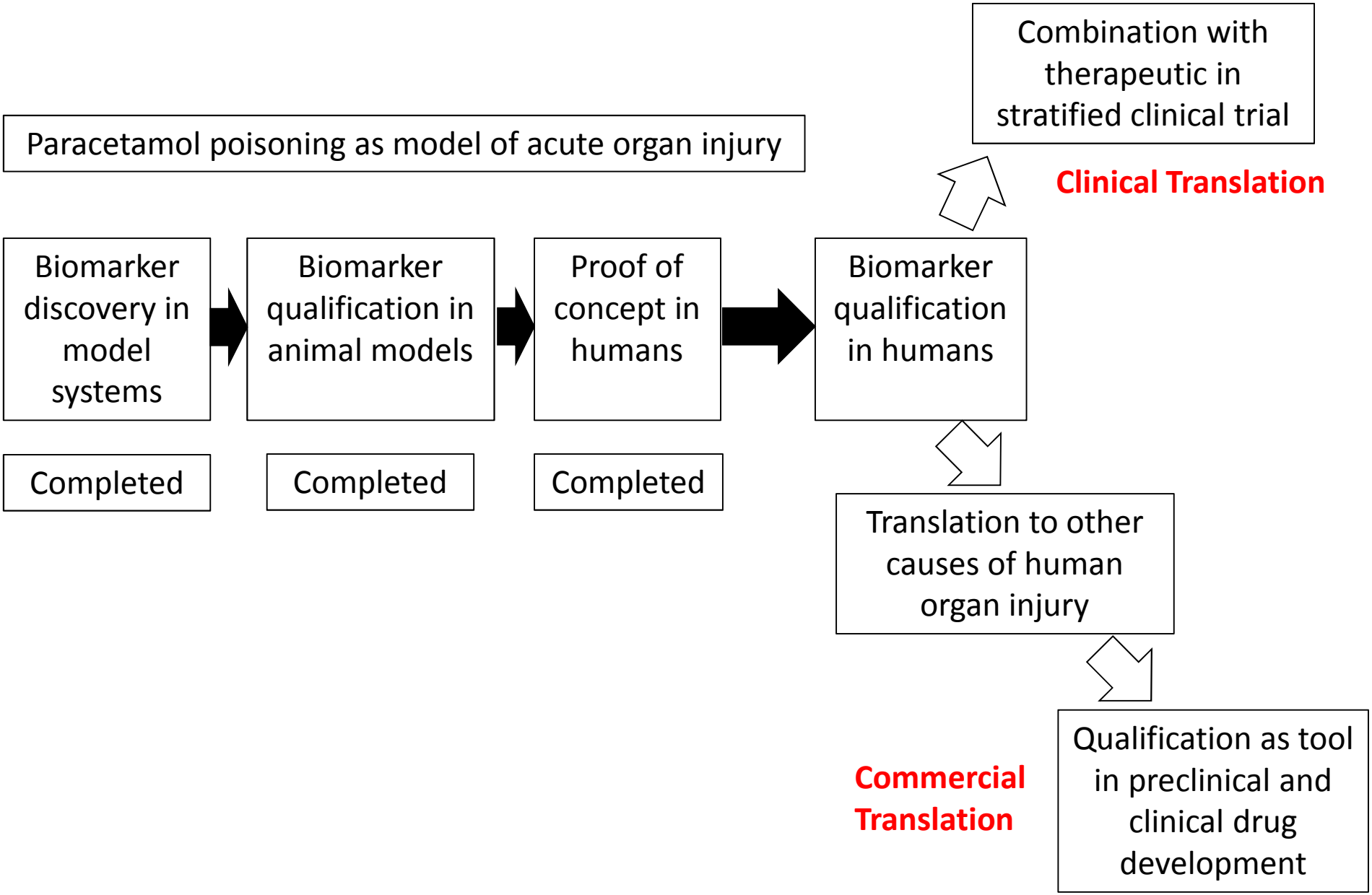






PLoS One – in press

Biomarker Developmental Pathway



Aladote® (PP-100)

Preventive treatment of acute liver failure in conjunction with acetaminophen poisoning

PledPharma develops Aladote® to counter the onset of acute liver failure caused by acetaminophen poisoning. Acetaminophen poisoning is one of the more common intoxications following deliberate or accidental overdose of drugs. Up to 50% of all serious poisonings occur unintentionally. The problem has increased in scale and the Swedish Medical Products Agency has recently decided to prohibit sales of acetaminophen outside pharmacies.

The degradation of acetaminophen in the liver has been found to be very harmful in high concentrations and can lead to acute liver failure. The existing treatment of acetaminophen overdosing (N-acetylcysteine) is effective if the affected patient seeks medical care within 8 hours after ingestion. For later arriving patients, there is currently no well-functioning treatment.

Aladote® has in relevant preclinical studies showed a good effect during the time window where treatment with N-acetylcysteine (NAC) is no longer functioning properly. PledPharma currently prepares the initiation of a clinical Phase II study.

PARACETAMOL QUESTION 3:

How should patients be treated?

Pediatrics. 1978 Nov;62(5 Pt 2 Suppl):898-903.

Acetaminophen Overdose: Incidence, Diagnosis, and Management in 416 Patients

Barry H. Rumack, M.D., *and* Robert G. Peterson, M.D., Ph.D.

From the Departments of Pediatrics, Medicine, and Pharmacology, and the Division of Clinical Pharmacology, University of Colorado Medical Center, Denver, and the Rocky Mountain Poison Center, Denver General Hospital

Intravenous N-acetylcysteine: the treatment of choice for paracetamol poisoning

L F PRESCOTT, R N ILLINGWORTH, J A J H CRITCHLEY, M J STEWART, R D ADAM, A T PROUDFOOT

British Medical Journal, 1979, **2**, 1097-1100

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Clinical Toxicology (2009) **47**, 81–88

ISSN: 1556-3650 print / 1556-9519 online
DOI: 10.1080/15563650802665587

REVIEW ARTICLE

Adverse reactions associated with acetylcysteine

E.A. SANDILANDS and D.N. BATEMAN

SNAP

Reduction of adverse effects from intravenous acetylcysteine treatment for paracetamol poisoning: a randomised controlled trial

D Nicholas Bateman, James W Dear, H K Ruben Thanacoody, Simon H L Thomas, Michael Eddleston, Euan A Sandilands, Judy Coyle, Jamie G Cooper, Aryelly Rodriguez, Isabella Butcher, Steff C Lewis, A D Bastiaan Vliegenthart, Aravindan Veiraiah, David J Webb, Alasdair Gray

Lancet 2014; 383: 697–704

With standard NAC regimen 31 of 100 had severe anaphylactoid reactions.

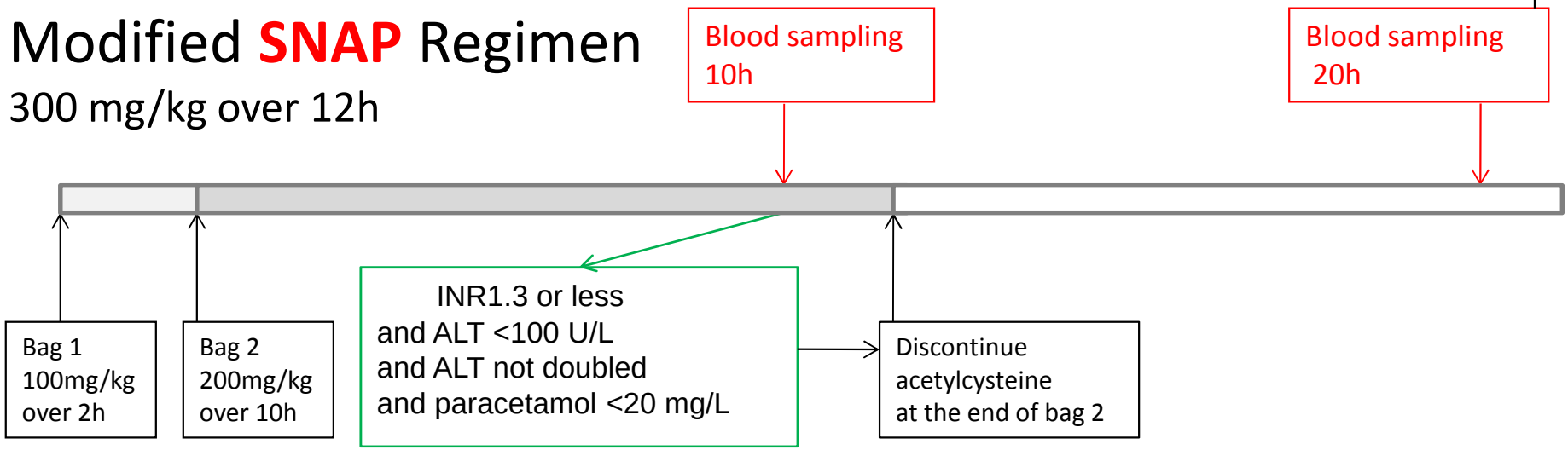
All 31 needed treatment of reaction and 26 needed interruption of NAC treatment

Current Regimen for Acetaminophen Poisoning

300 mg/kg over 21h

Modified SNAP Regimen

300 mg/kg over 12h



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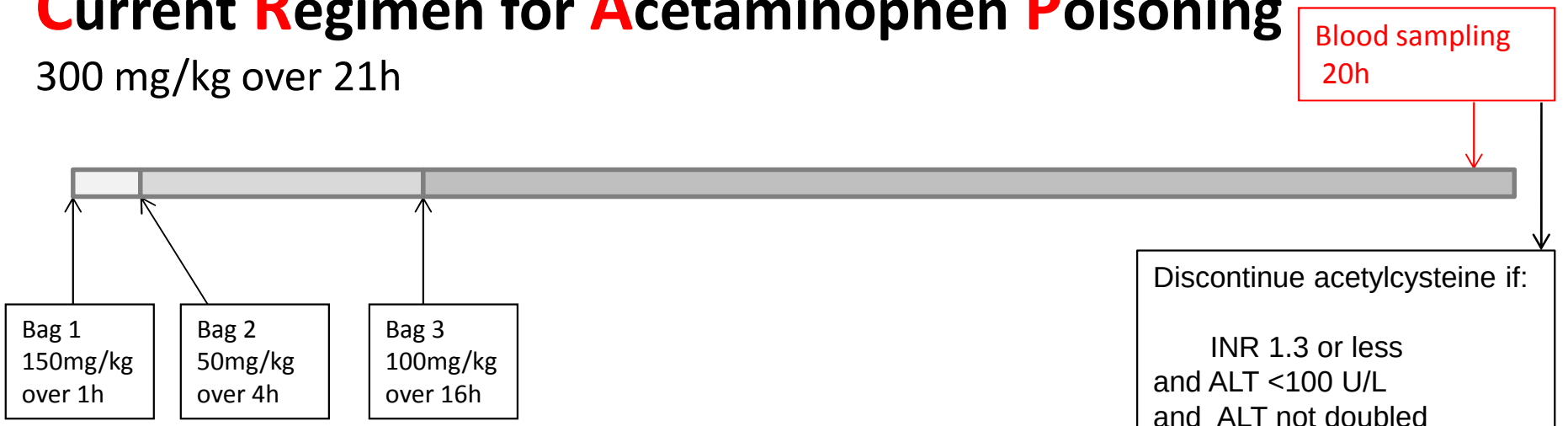
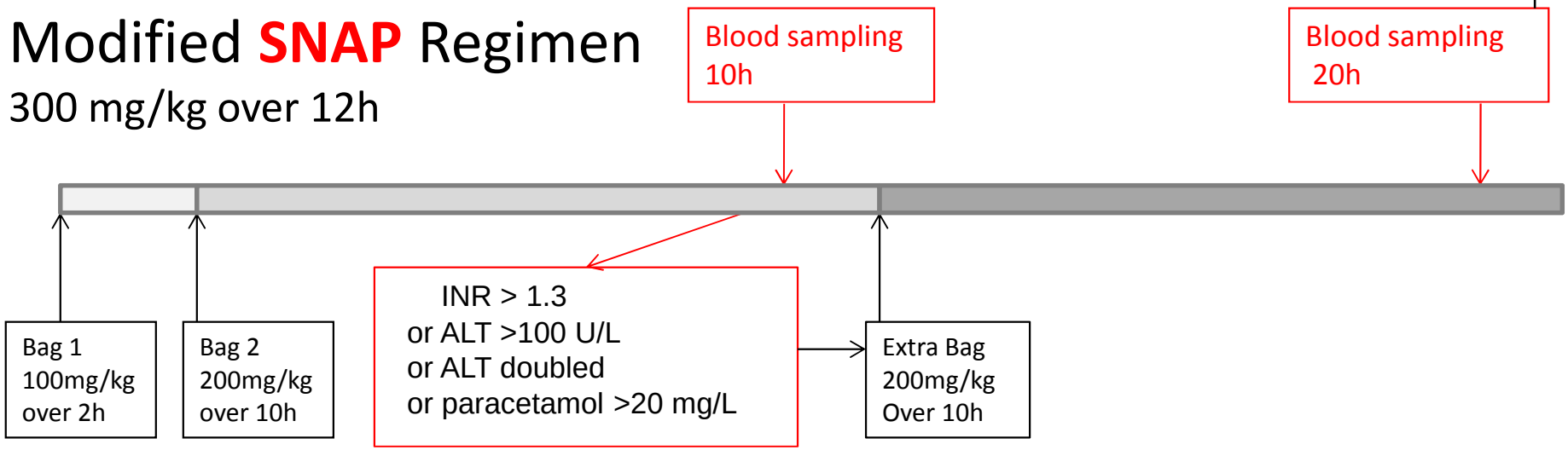
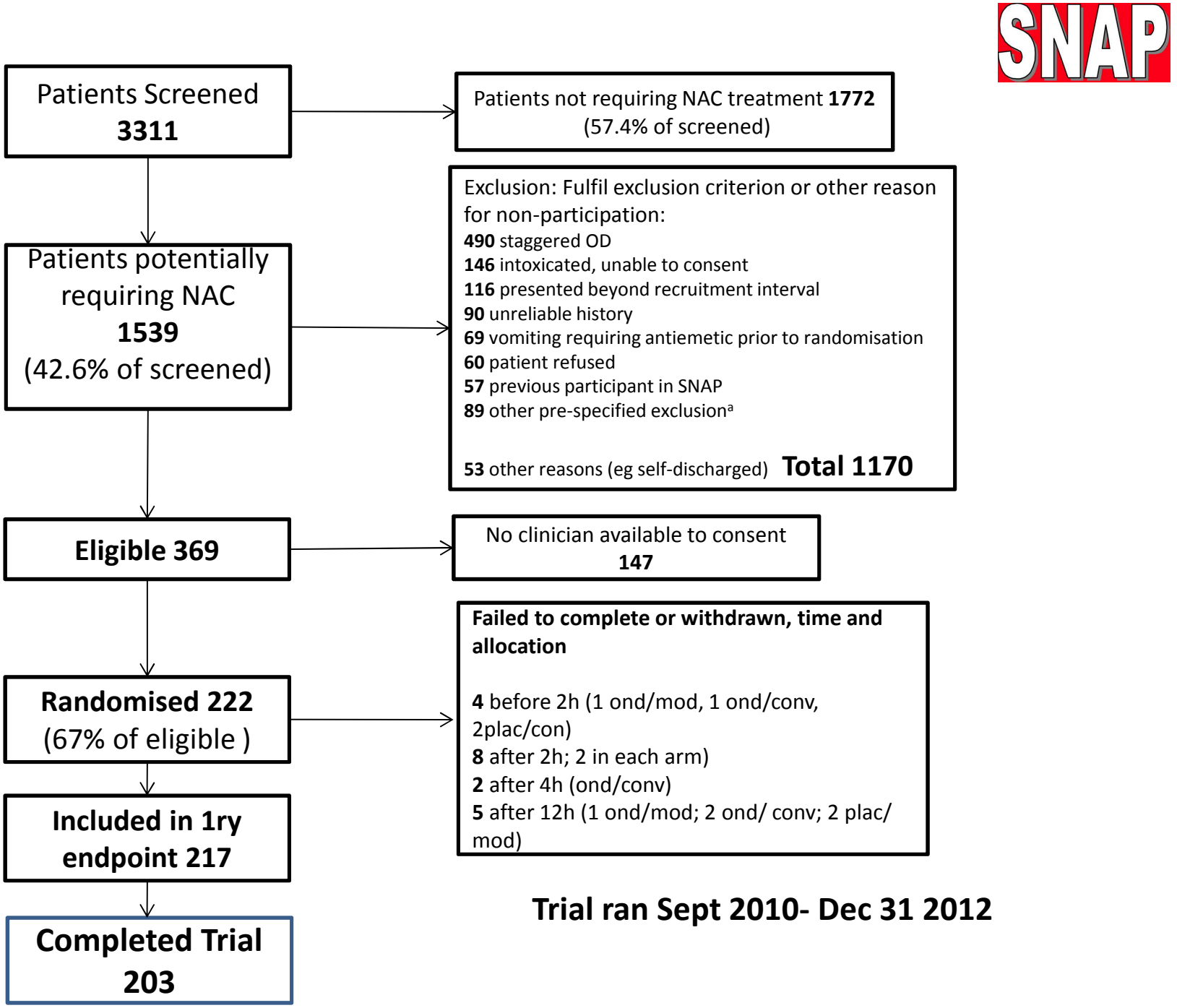


Modified SNAP Regimen

300 mg/kg over 12h





Trial ran Sept 2010- Dec 31 2012

Trial Safety

Anaphylactoid reaction:
Grade 3 responses – interruption or medication
(proportional odds regression)

Comparison	Treatment Group	Number with outcome	Total number	Odds Ratio	97.5% confidence interval	P-value
NAC Regimen	Modified	5	108	0.2	0.1-0.4	<0.0001
	Conventional	31	100			

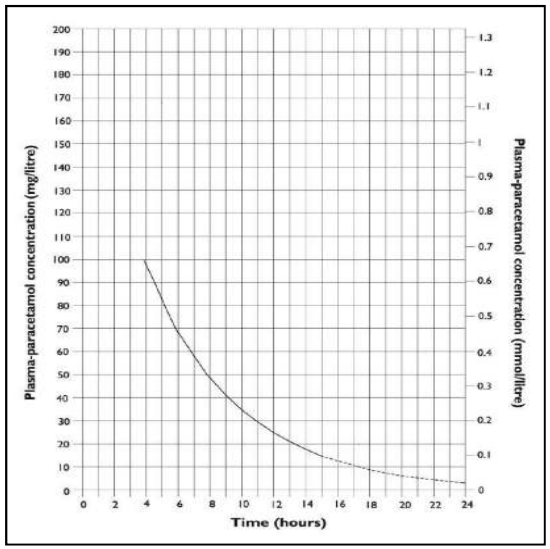
Real world data – Royal Infirmary of Edinburgh

Standard 21h regimen group

18 Months

Admissions n=985

Treated n=786



Modified 12h regimen group

18 Months

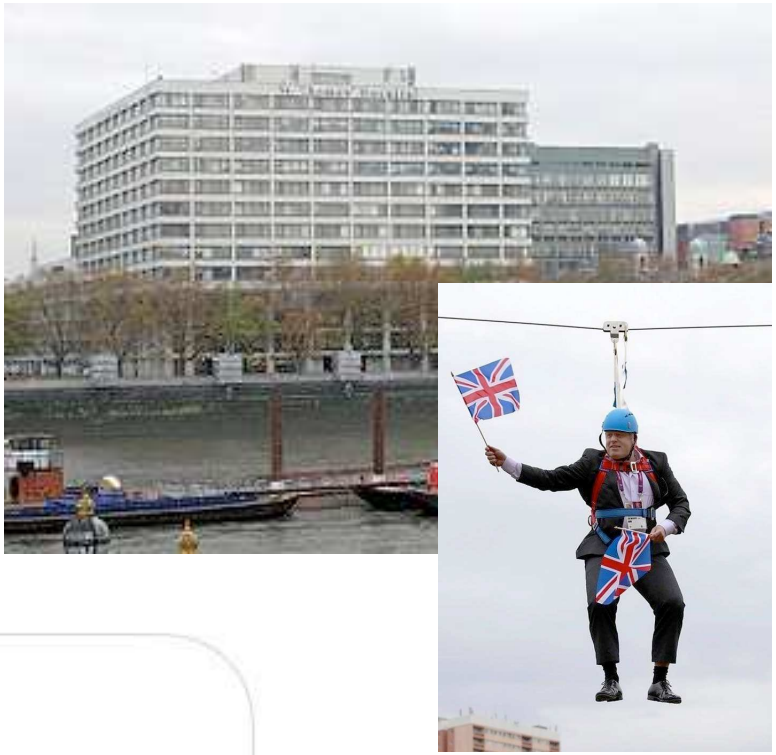
Admissions n=984

Treated n=855

Not just Edinburgh

Newcastle

London



TOXBASE.org

Real world safety

	Standard 21h Regimen 18 months N=786		Modified 12h Regimen 18 months N=855		Absolute Reduction (95% CI)
	n	%	n	%	
Anaphylactoid reactions	88	11	12	2	**10 (8 - 12)**

Only need to treat 10 people to prevent one anaphylactoid reaction

Real world safety – repeat presenters (N=41)

	Standard 21h Regimen 85 treatments		Modified 12h Regimen 125 treatments		Absolute Reduction (95% CI)
	n	%	n	%	
Anaphylactoid reactions	8	9	1	1	**9 (3 - 17)**

Trial Efficacy

50% rise in ALT at 20.25 h

Present in 22/201 (10.9%)

NAC modified v conventional OR **0.603**, 97.5% CI **0.199-1.831**

miR-122 in subset Edinburgh patients (124) (median and IQR)

NAC modified 1.1 (0.4-2.4) v conventional 0.5 (0.2-3.4) **p = 0.789**

Doubling ALT (19/201)

NAC modified (10) v conventional (11) OR 0.653 97.5 % CI 0.195-2.183 **p=0.4283**

Extra acetylcysteine beyond 20.25 h in 17 patients

NAC regime, OR 2.2 (97.5% CI 0.6 to 7.8) **P=0.18**

Trial Efficacy

50% rise in ALT at 20.25 h

Present in 22/201 (10.9%)

There is no signal of reduced efficacy

Extra acetylcysteine beyond 20.25 h in 17 patients

NAC regime, OR 2.2 (97.5% CI 0.6 to 7.8) **P=0.18**

Real world efficacy

Discontinue acetylcysteine if: INR 1.3 or less and ALT <100 U/L and ALT not doubled	**Standard 21h Regimen N=786** **n %**	**Modified 12h Regimen N=855** **n %**	**Absolute Reduction (95% CI)**
Acute liver injury			
Treatment beyond 21h	81 10	88 10	**0 (-3 – 3)**
ALT > 1000 U/L	23 3	24 3	**0 (-1.5 – 1.8)**

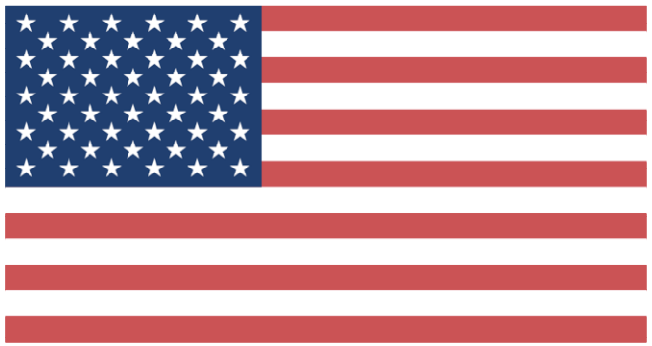
Real world efficacy

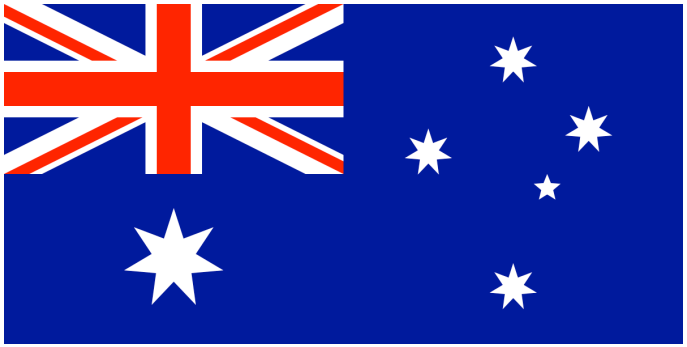
	Standard	Modified 12h	
ALT > 1000 U/L	22 3	24 3	(-1.5 – 1.8)

There is no signal of reduced efficacy

Single overdose above 150 line

	Standard 21h Regimen N=277 n %	Modified 12h Regimen N=267 n %	Absolute Reduction (95% CI)
Acute liver injury			
ALT > 1000 U/L	11 3	11 3	**0 (-4 – 3)**

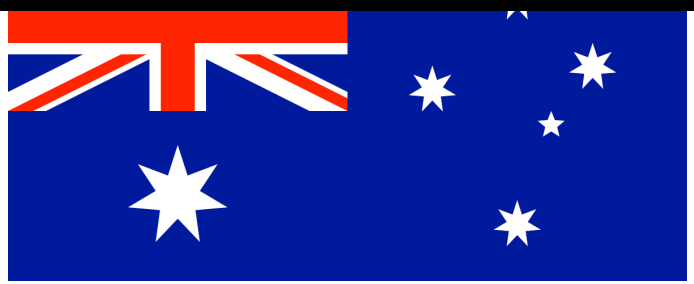




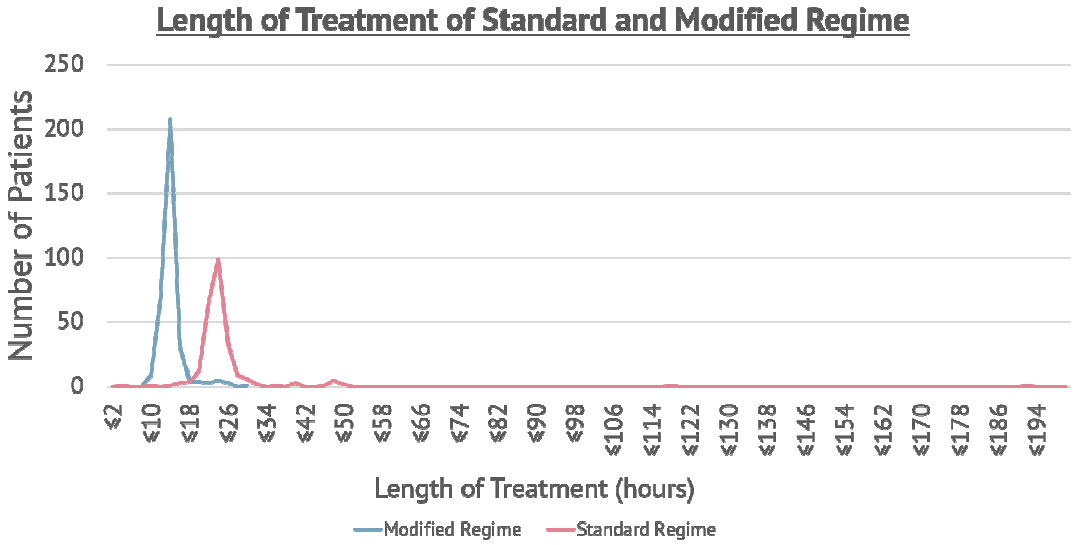
Single overdose above 150 line

	Standard	Modified 12h	Absolute

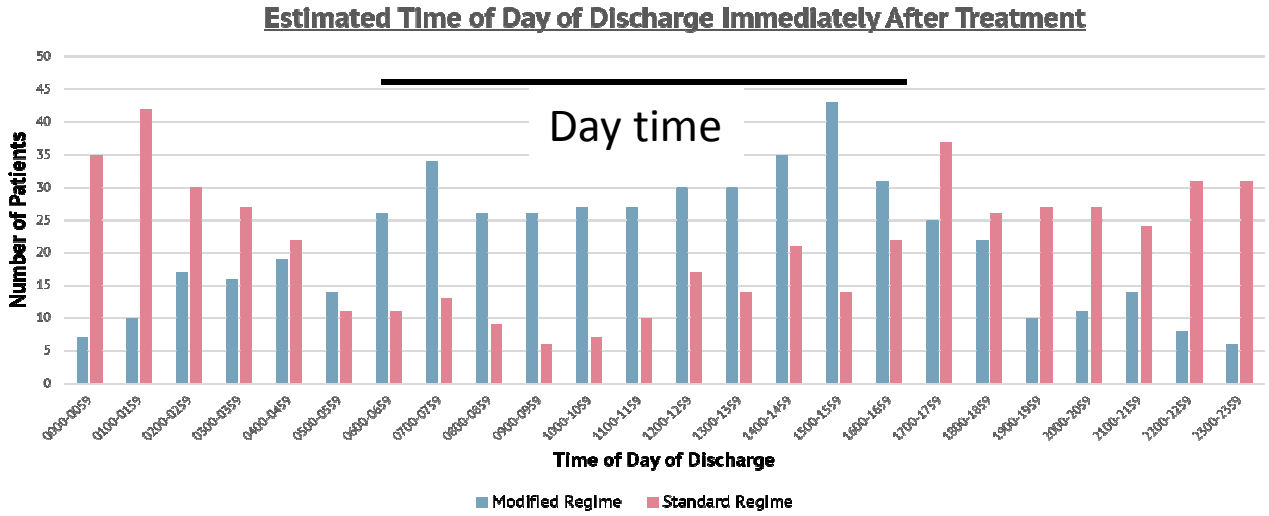
There is no signal of reduced efficacy



Health economic benefits



US $13 million saved per year in UK



Conclusions

12h regimen is associated with:

- **Significantly less adverse effects**
- **No increase in liver injury**
- **Current 10h blood rule out criteria appear safe**

Conclusions

Biomarker tool kit can stratify patients

Activity is underway with regard to point of care / clinical assays

New NAC regimen is safer and shorter

University of Edinburgh
Bastiaan Vliegenthart
Wilna Oosthuyzen
Emma Morrison
Olivia Matthews
Kathleen Scullion
Bean
Chunmin Wei
Ken Simpson
Professor David J Webb
Professor Nick Bateman
Matthew Bailey
Professor Alasdair Gray
Till Bachmann
Holger Schulze

Qiagen
Jonathan Shaffer
Eric Lader

CDSS University of Liverpool
Professor Kevin Park
Dr Dan Antoine
Dr Chris Goldring
Phillip Starkey-Lewis
Vivien Platt
Jack Sharkey

NPIS Newcastle
Ruben Thanacoody
Professor Simon Thomas

St Thomas' Hospital London
Dr Paul Dargan
Dr David Wood

Heriot Watt University
Maïwenn Kersaudy-Kerhoas

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