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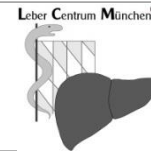
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Transforming monocytes into hepatocyte surrogates

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CHALLENGES OF IDIOSYNCRATIC DILI (iDILI)

- Rare occurrence (e.g. 1 in 10.000)
 - Numerous individual factors that influence susceptibility
 - May be difficult to diagnose and especially to identify the causative agent in polymedication
 - Lack of experimental models
-

NEED: Models for iDILI

Positive test supporting the diagnosis and causality assessment



MONOCYTE DERIVED HEPATOCYTE-LIKE CELLS (MH CELLS)

BACKGROUND

- Monocytes seem to be important for hepatic repair in models of acute liver injury (1)
- Monocytes may be capable to transform into hepatocytes(2)
- Previous data suggest that cells with hepatocyte-like functions can be generated from peripheral monocytes (3, 4)

- 1) Hogaboam CM, Bone-Larson CL, Steinhauser ML, et al. Am J Pathol. 2000 Apr;156(4):1245-52.
- 2) Stadtfeld M, Graf T. Development. 2005 Jan;132(1):203-13. Epub 2004 Dec 2.
- 3) Ruhnke M, Ungefroren H, Nussler A. Gastroenterology. 2005 Jun;128(7):1774-86.
- 4) Benesic A, Gerbes AL et al. Lab Invest Jun;92(6):926-362; 2012



MONOCYTE DERIVED HEPATOCYTE-LIKE CELLS (MH CELLS) GENERATION

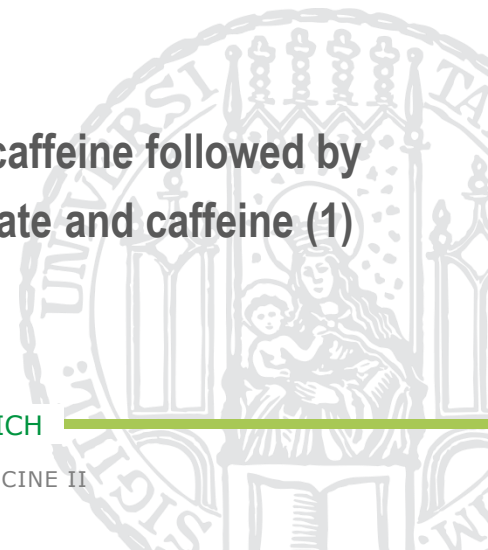
ISOLATION:

- 20ml EDTA-plasma
- Gradient centrifugation
- Adherence separation of monocytes from EDTA-plasma sample

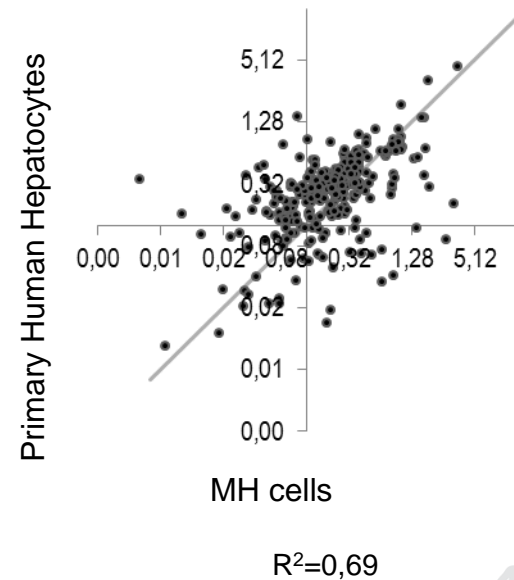
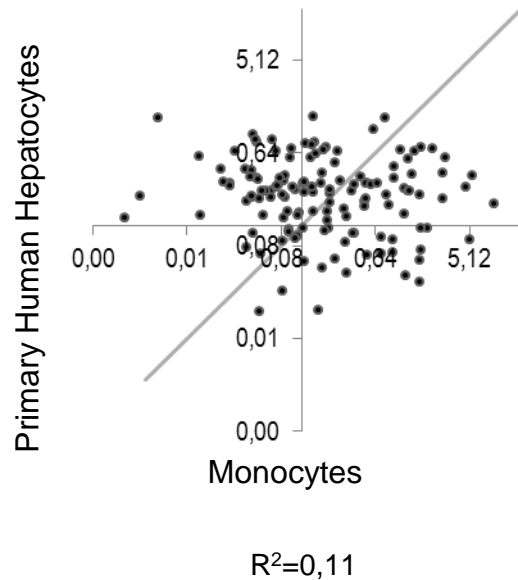
CULTURE:

- Cultivation in presence of adenosine, IL-3, M-CSF, deoxycholate and caffeine followed by medium containing EGF, FGF4, heparin, glucagon, insulin, deoxycholate and caffeine (1)

1) Benesic A, Gerbes AL et al. Lab Invest Jun;92(6):926-362; 2012; US Patent number 8858934



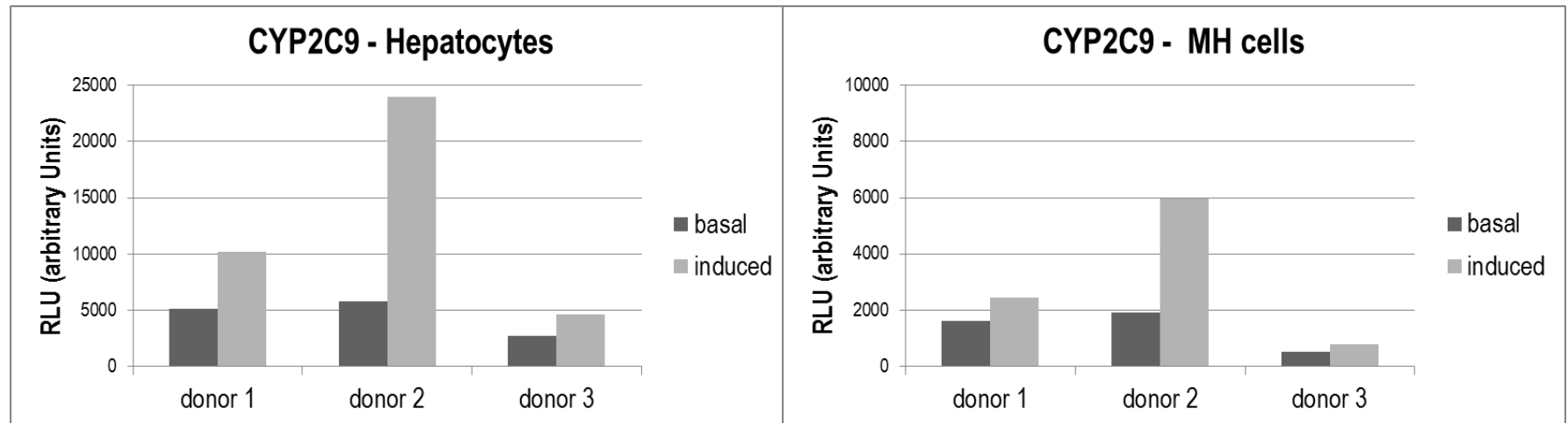
MONOCYTE DERIVED HEPATOCYTE-LIKE CELLS (MH CELLS) GENE EXPRESSION COMPARED TO HEPATOCYTES



Gene expression profiles of monocytes (left) and MH cells (right) compared to primary human hepatocytes (PHH) from the same donor



MONOCYTE DERIVED HEPATOCYTE-LIKE CELLS (MH CELLS) DONOR CHARACTERISTICS – EXAMPLE: CYP2C9

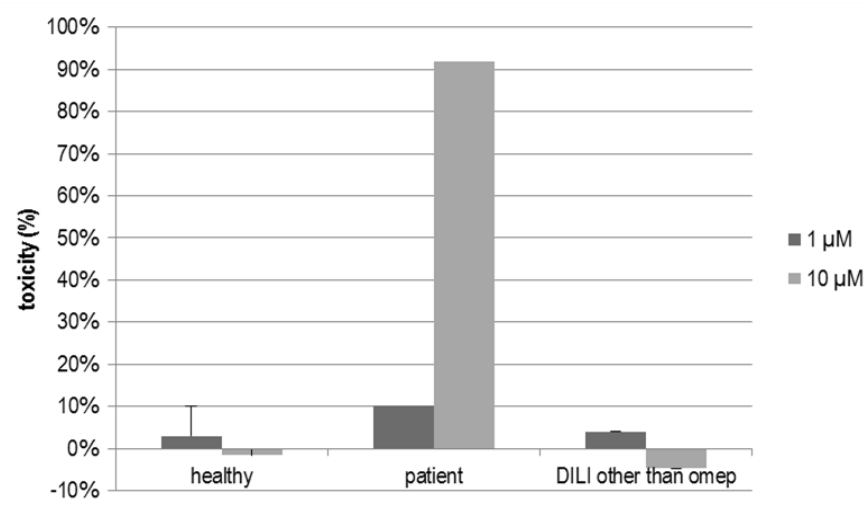
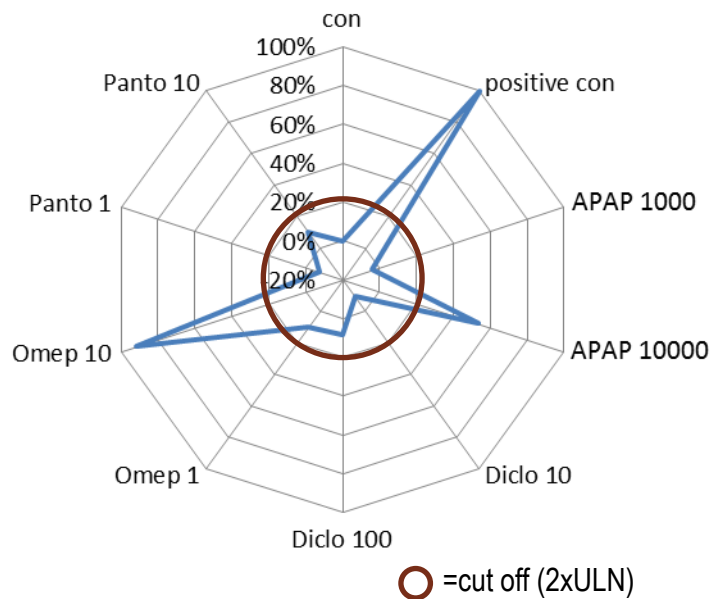


CYP450 activities and induction levels of primary hepatocytes are reflected by MH cells from the same donor



MH CELLS FROM PATIENTS WITH iDILI

CASE: iDILI BY OMEPRAZOLE

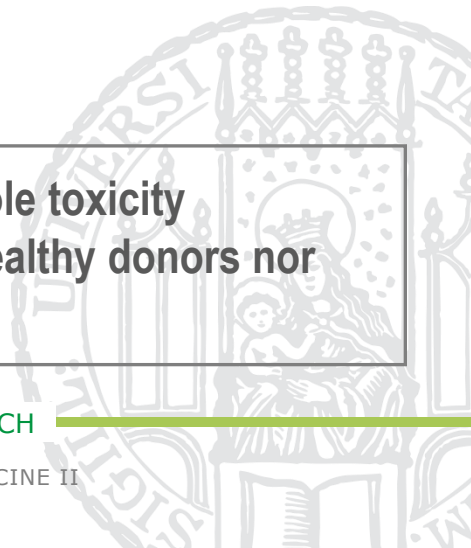


Abbreviations:

- con: vehicle control
- positive con: lysis with TWEEN20
- APAP: acetaminophen (µM)
- Diclo: diclofenac (µM)
- Omeprazole: omeprazole (µM)
- Pantoprazole: pantoprazole (µM)

- MH cells of the patient exhibit omeprazole toxicity

- No omeprazole toxicity in MH cells of healthy donors nor patients with iDILI by another drug



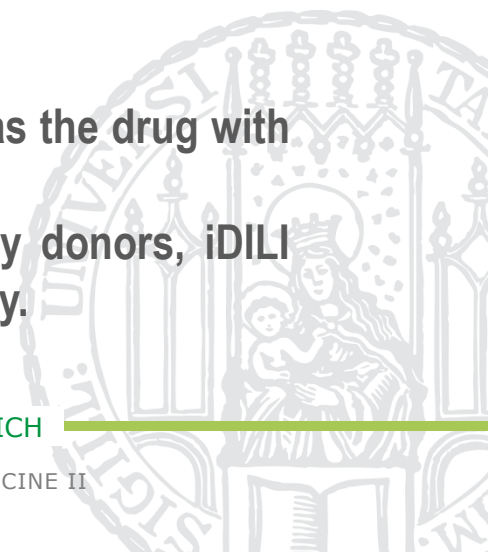
RESULTS

SPECIFICITY OF MH CELL TESTING

MH	healthy	iDILI diclo	iDILI other than diclo	non DILI
≥2	0	4	0	0
<2	81	0	27	23

MH testing is positive for diclofenac in 4 iDILI cases with diclofenac as the drug with the highest causality likelihood.

No diclofenac toxicity is observed in MH cells derived from healthy donors, iDILI patients with another drug as cause and non DILI patients, respectively.



MH CELLS

PILOT STUDY IN PATIENTS WITH ACUTE LIVER INJURY

Study aim:

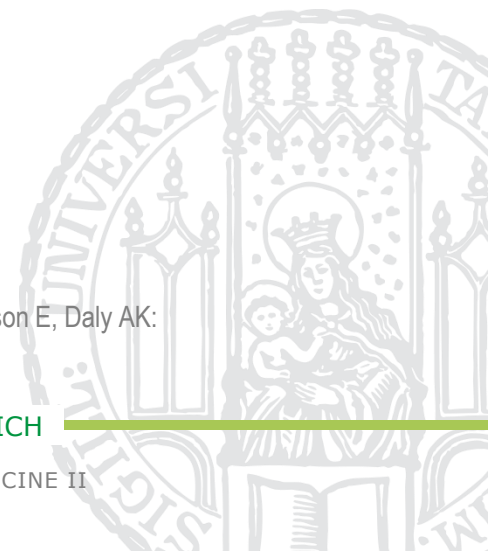
- Investigation of potential individual toxicity response of MH cells from iDILI patients in comparison to MH cells from patients with acute liver injury of other origin

Methods:

- Patients treated with at least one drug and acute liver injury according to (1):
ALT \geq 5X ULN or AP \geq 2X ULN or ALT \geq 3X ULN and Bili \geq 2X ULN
- Diagnostic Workup: laboratory testing, imaging, histology (where available), RUCAM-score, drug signature (e.g. LiverTox website (2))
- MH cell generation, toxicity testing, data analysis

ClinicalTrials.gov Identifier: NCT02353455

- 1) Aithal GP, Watkins PB, Andrade RJ, Larrey D, Molokhia M, Takikawa H, Hunt CM, Wilke RA, Avigan M, Kaplowitz N, Bjornsson E, Daly AK: Clin Pharmacol Ther. 2011 Jun;89(6):806-15. doi: 10.1038/clpt.2011.58.
- 2) Hoofnagle JH, Serrano J, Knoblen JE, Navarro VJ. Hepatology. 2013 Mar;57(3):873-4. doi: 10.1002/hep.26175



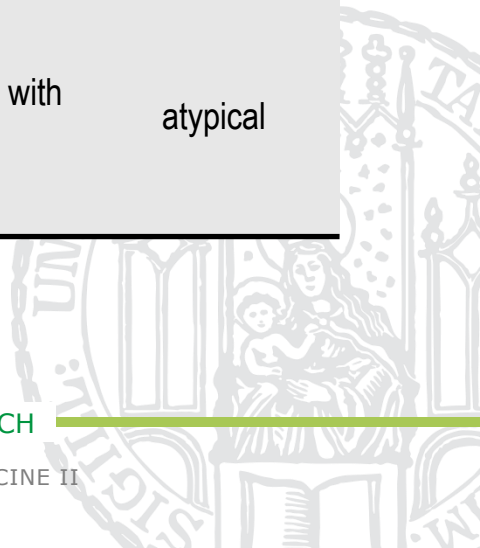
DIAGNOSIS OF iDILI

exclusion of other causes

drug signature

Classification for every drug

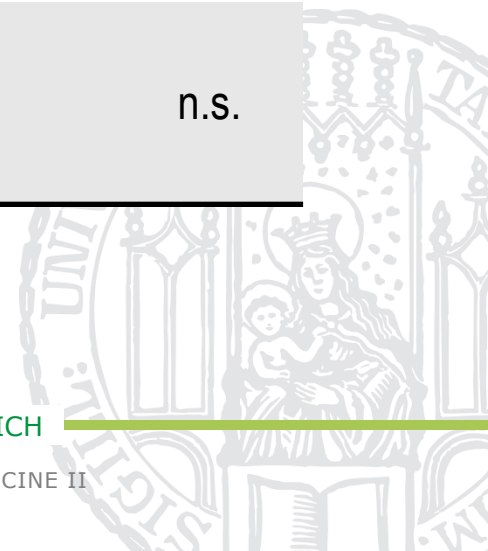
diagnosis	iDILI			non DILI	
classification:	„definite“	„highly likely“	„probable“	„possible“	„unlikely“
other causes of liver injury	definitely excluded	definitely excluded	unlikely	probable	highly probable
drug signature	typical	atypical OR comedication with compatible signature	atypical AND comedication with compatible signature	compatible with iDILI	atypical



RESULTS: PATIENT CHARACTERISTICS

31 patients with iDILI and 23 with other causes for acute liver injury

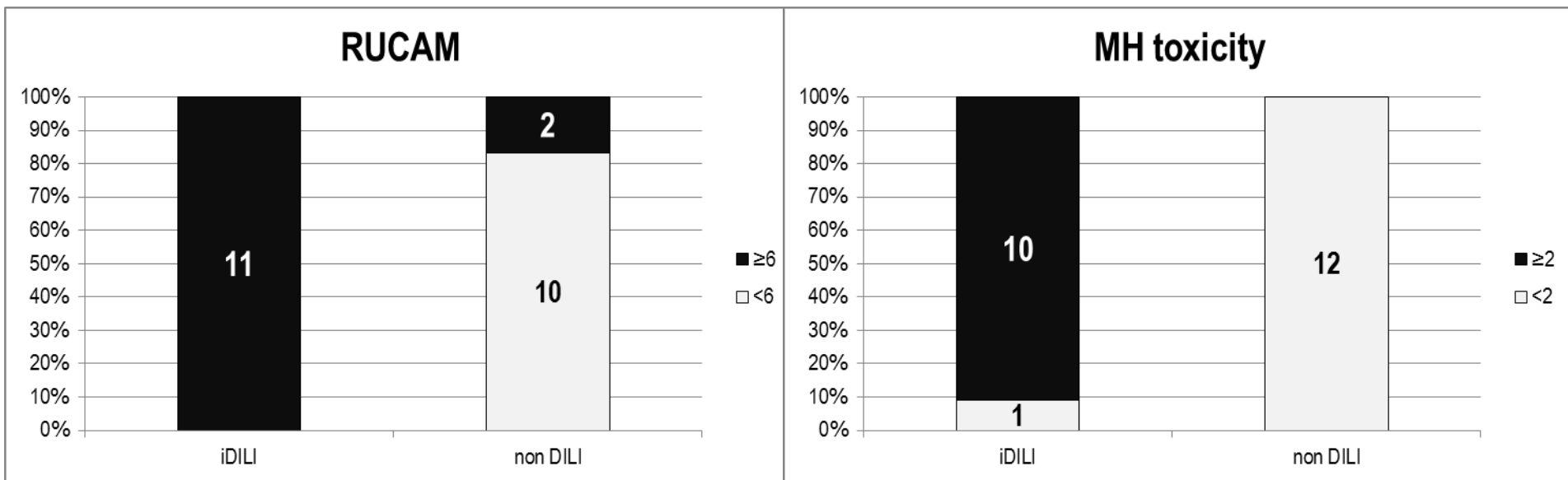
	iDILI (n=31)	non DILI (n=23)	P
gender	15 female (48%) 16 male (52%)	9 female (39. %) 14 male (61%)	n.s.
ethnicity	30 caucasian (97%) 1 hispanic (3%)	23 caucasian (100%)	n.s.
pattern	22 hepatocellular (71%) 2 mixed (7%) 7 cholestatic (23%)	13 hepatocellular (57%) 0 mixed (0%) 10 cholestatic (43 %)	n.s.



RESULTS: DRUGS WITH HIGHEST CAUSALITY LIKELIHOOD IN IDILI

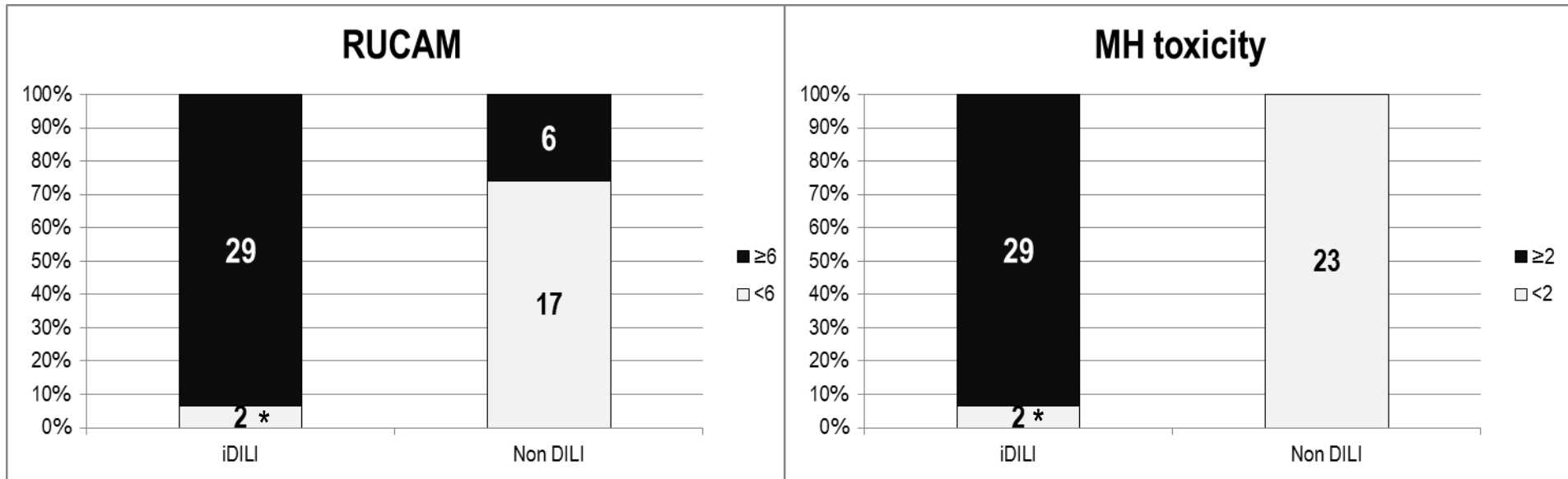
Class	n	drug	n
NSAID	8	diclofenac	4
		metamizol	3
		indomethacin	1
ORAL ANTICOAGULANT	4	phenprocoumon	4
ANTITHYROID	2	carbimazole	2
ANTI-INFECTIVE	2	ciprofloxacin	1
		piperacillin+ tazobactam	1*
IMMUNEMODULATOR	2	glatirameracetat	1
		pembrolizumab	1*
ANTIPSYCHOTIC	2	olanzapine	1
		fluspirilene	1
PROTON PUMP INHIBITOR	2	omeprazole	1
		pantoprazole	1
antihistamine	1	cetirizin	1
antifibrotic	1	4-potassium aminobenzoate	1
low-molecular weight heparin	1	enoxaparin	1
phosphodiesterase 5-inhibitor	1	tadalafil	1
serotonin-receptor antagonist	1	sumatriptan	1
oral contraceptive	1	ethinylestradiol + levonorgestrel	1
tyrosin-kinase inhibitor	1	sunitinib	1
vitamin A analogue	1	acitretin	1
muscle relaxant	1	flupirtin	1

RESULTS: DRUG WITH HIGHEST CAUSALITY LIKELIHOOD RUCAM AND MH TOXICITY IN UNEQUIVOCAL CASES



- RUCAM ≥ 6 is found in 2 non DILI cases
- MH testing is negative in 1 case with unequivocal iDILI

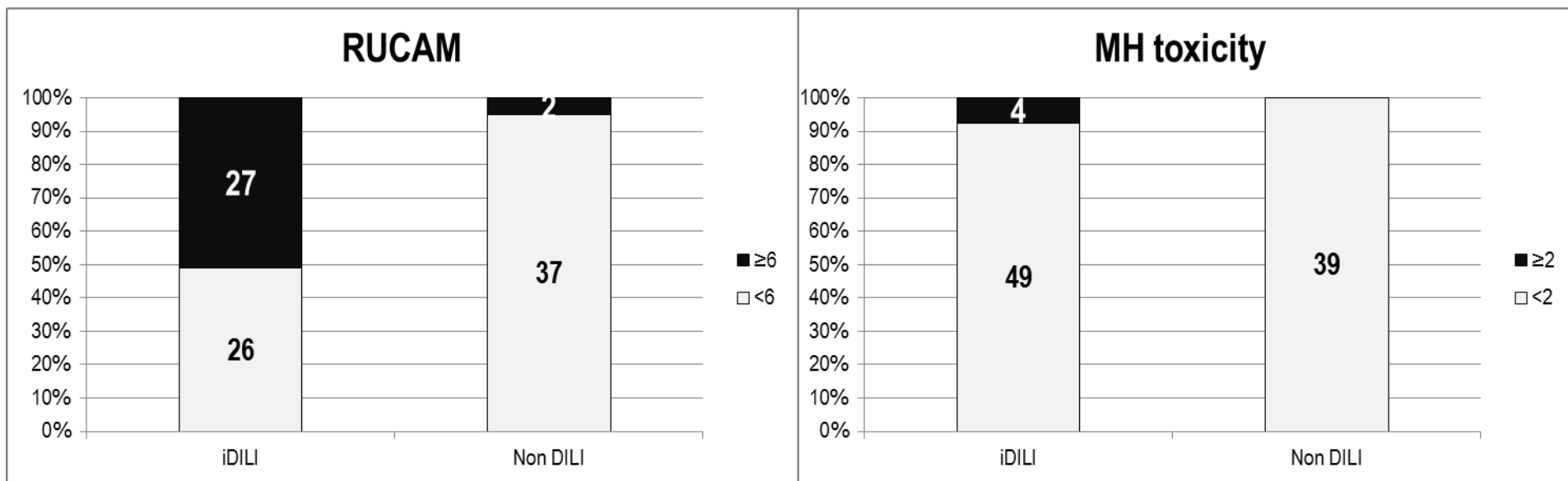
RESULTS: DRUG WITH HIGHEST CAUSALITY LIKELIHOOD RUCAM AND MH TOXICITY IN TOTAL STUDY POPULATION



- RUCAM ≥ 6 is found in 29/31 iDILI cases and 6/23 non DILI cases
- MH testing is negative in 2 iDILI cases, no positive MH testing in non DILI cases

*different cases

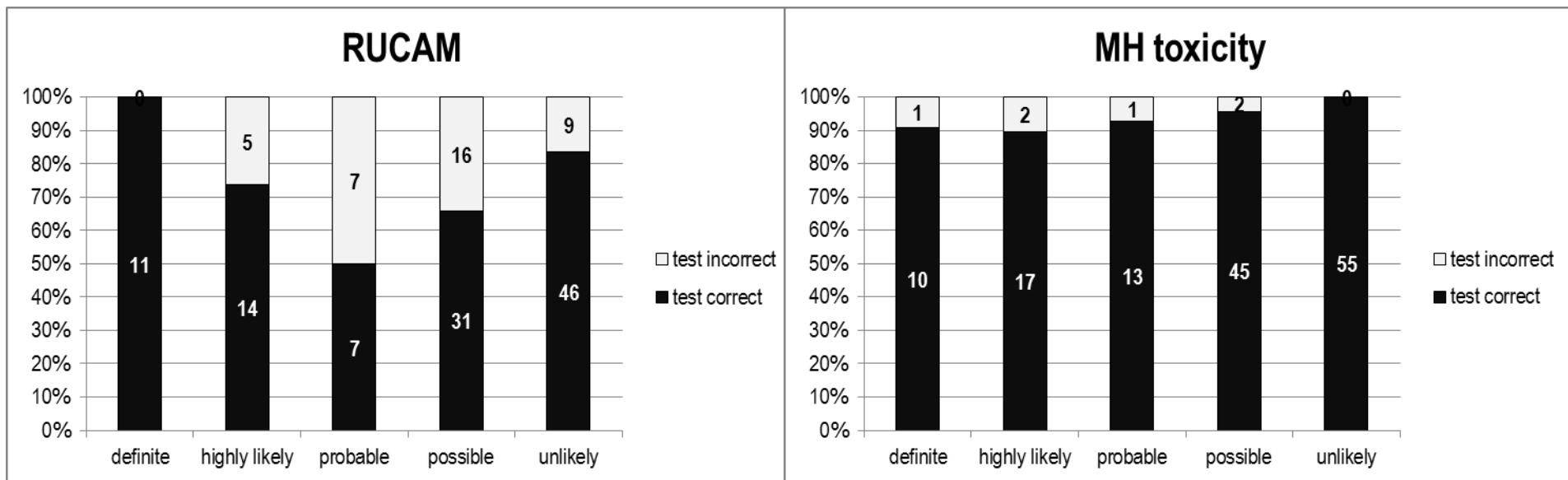
RESULTS: COMEDICATIONS RUCAM AND MH TOXICITY IN TOTAL STUDY POPULATION



Test results for comedICATIONS in all iDILI (n=31) and non DILI (n=23) cases (up to 4 drugs per patient):

- RUCAM is ≥ 6 for 27/53 comedICATIONS in iDILI cases and 2/39 non DILI cases
- MH testing is positive for 4 comedICATIONS in iDILI cases, no positive results in non DILI cases

RESULTS: ALL DRUGS IN TOTAL POPULATION TEST RESULTS COMPARED TO CAUSALITY LIKELIHOOD



- RUCAM yields a relevant proportion of false results for drugs with intermediate causality likelihood
- MH testing shows stable results independent on causality likelihood

SUMMARY

- Our data suggest that monocytes can acquire some hepatocyte properties in vitro and seem to reflect donor specific characteristics
- In this pilot study toxicity was higher in MH cells derived from iDILI patients compared to patients with non DILI acute liver injury or healthy donors
- MH cells offer the possibility to assist with diagnosis of iDILI and causality assessment

OUTLOOK

- Ongoing research further characterises the model using Omics-technologies
- Further data are needed from patients who tolerate potential iDILI drugs

