Blood-based IVDMIAs and longitudinal molecular marker analysis in the rat chronic constrictive injury model: disease biology and benchmark profiling.

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Introduction:

Neuropathic pain (Neup) is a term used to define a heterogeneous group of chronic pain conditions which may arise as a consequence of a lesion or disease affecting the somatosensory system[1]. It reduces quality of life and poses a huge economic burden to the health system and society.

Along with dual serotonin and noradrenaline reuptake inhibitors the anticonvulsant Pregabalin (PGB) is the recommended first line of treatment in Neup. In the majority of patients, PGB is poorly efficacious (NNT=4) and often associated with undesirable side-effects. There is therefore, a dire need to develop novel more efficacious treatments with fewer side effects. To serve this need, we have developed an approach to understand better the mechanistic aspects of neuropathic pain biology and of PGB response in rodents.

Aim:

Measure longitudinal transcriptional changes, in whole blood, directly coupled to the phenotypic profile to build blood-based *in vitro* diagnostic multivariate index assays (IVDMIAs). Enabling assessment of intracellular mRNA changes that arise as a result of the disease pathology, building reproducible responder/non-responder profiles to drug treatment in the CCI model of neuropathy, as well as providing reliable blood read-outs predictive of drug response prior to treatment initiation.

Methods:

The CCI model was used to induce neuropathic pain. Mechanical allodynia was measured using von-Frey hairs and the up-down method described by[2,3]. Blood samples for IVDMIA analysis were collected from each animal, at Day0 (Baseline), Day20 (Disease biology) and Day31 (post-PGB treatment; Figure1). The experiment was repeated twice to ensure profile reproducibility. RNA copy numbers for a panel of 200 markers were measured with the nCounter platform (Nanostring Technologies). Principle component analysis (PCA) was used to reveal the best discriminating patterns between animal groups.

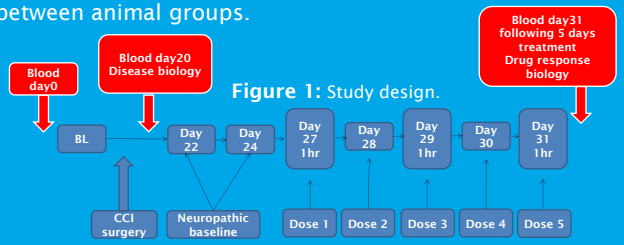


Figure 1: Study design.

It is much more important to know what kind of patient has a disease, than to know what kind of disease a patient has- William Osler.

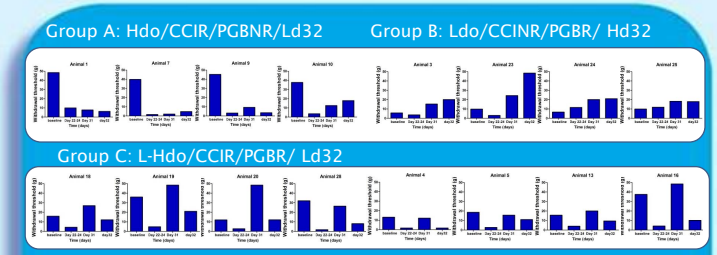


Figure 2: Three distinct behavioural phenotypes were identified;

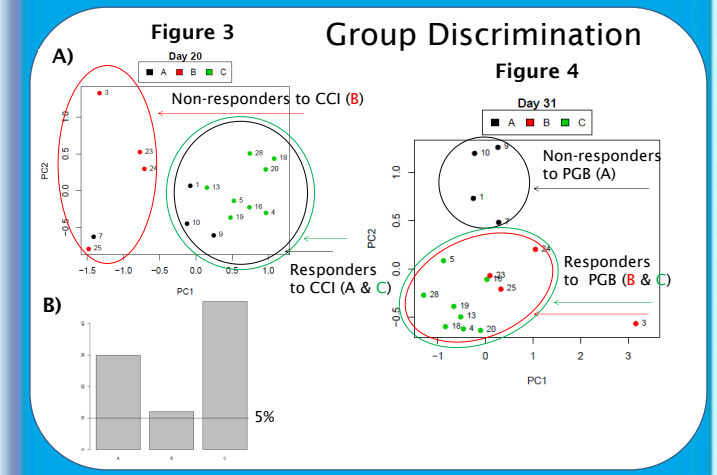


Figure 3: A) Group discrimination in CCI. IVDMIA reliably discriminates responders and non-responders to the ligation (disease pathology). **B)** Of the 200 profiled transcripts , approx. 60 were altered by the model-Disease biology.

Figure 4: Treatment discrimination in CCI. IVDMIA reliably discriminates responders and non-responders to PGB.

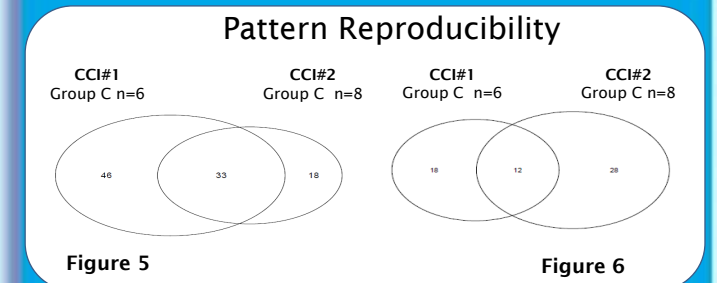
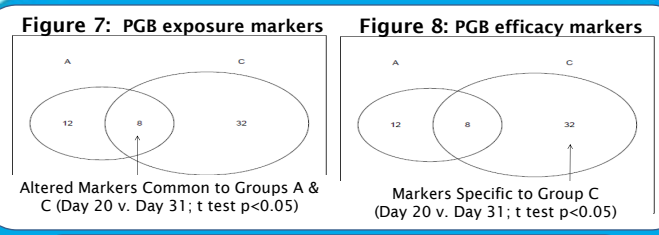


Figure 5: IVDMIAs identify reproducible pattern of disease biology: 33 of the 51 markers altered in CCI#2 were altered in CCI#1; 65% reproducibility; $P=2.3164\times10^{-5}$.

Figure 6: IVDMIA identifies reproducible molecular markers resulting from PGB treatment: 12 of the 40 markers altered in CCI#2 were also altered in CCI#1; After subtracting exposure markers, this leaves a core of 8 markers associated with PGB efficacy; P=0.00502.



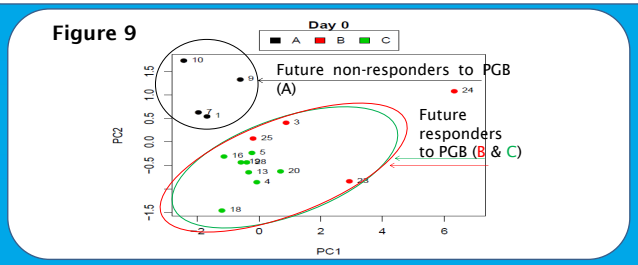


Figure 9: Predictive value of IVDMIA at Day 0: Future responders and non-responders clearly identified prior to CCI surgery and treatment initiation

Marker	mean A (n=4) d20 (log2 data)	t test A v. C	mean C (n=8) day20 (log2 data)	Comments
KIT	4.974611791	0.011	4.371627001	c-KIT involved in the development of persistent pain[4]
GLRX5	3.406196131	0.025	2.666931443	
VDAC1	6.972372033	0.033	6.81489253	VDAC antagonists block neuropathic pain in CIN model[5]
SOD1	9.185707972	0.035	8.920338072	Overexpression of SOD1 increases neuropathic pain in sciatic nerve injury mouse model[6]
PLA2G7	7.339501855	0.042	7.577449626	Act as PAF antagonist to block neuropathic pain[7]

Table 1: Predictive value of IVDMIA at Day 20: Drug response predictive markers and their biological relevance

Conclusion:

Blood based IVDMIAs can generate reproducible disease state, PGB exposure/efficacy biomarker patterns and carry predictive value for future drug response; Therefore they are useful tools to improve the R&D process and potentially assist patient management. miRNA profile should also be established and integrated in the IVDMIAs for best understanding of transcriptional networks involved in disease and drug response

1) **Efficacy:** transcription regulation of IL12; Redox status (CAT, NOSIP; GPX1, MGST1, MGST3, FAM213A)
2) **Exposure:** PROK2, CXCR4, CTSS, NCOA1
3) **Predictive Day 0:** ZC3H12A, TGFB1 (pro-inflammatory tone in group A; IL-6, IL12B; CALR)
4) **Predictive Day 20:** KIT, VDAC1, SOD1, PLA2G7

The PGB efficacy signature is consistent with NK and Th1 cell function modulation and is supported by a number of reports documenting the involvement of ROS production, cellular redox status and the immune system in the control of neuropathic pain [8, 9]

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References: 1) Jensen et al., Pain. 2011, 152:2204-5. 2) Dixon, Ann Rev Pharmacol Toxicol. 1980, 20:441-62. 3) Chaplan et al., J Neurosci Methods. 1994, 53:55-63. 4) Sun et al., Pain. 2009, 144:178-86. 5) Zheng et al., Exp. Neuro. 2011, 232:154-61. 6) Kotulska et al., J. Neuro. Res. 2006, 84:1091-97. 7) Motoyama et al., Eur. J. Pain. 2013, 17:1156-67. 8) Jang et al., Anesthe. - Analge. 2012, 115:830-6. 9) Xu et al., Antiox. & Redox Signal. 2010, 13:27-37