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ASX: NOX

Noxopharm Limited

ABN 50 608 966 123

Registered Office:
Suite 1 Level 6
50 Queen St
Melbourne VIC 3000
Australia

Operational Office:
Suite 3, Level 4
828 Pacific Highway
Gordon NSW 2072
Australia

Board of Directors
Mr Peter Marks
Chairman
Non-Executive Director

Dr Graham Kelly
Chief Executive Officer
Managing Director

Dr Ian Dixon
Non-Executive Director

ASX Limited
20 Bridge Street
SYDNEY NSW 2000

NYX-104 DELIVERS KEY PROOF-OF-CONCEPT IN ANIMAL MODEL OF STROKE

- **NYX-104 being developed as drug to limit extent of brain damage in response to trauma**
- **NYX-104 shown to deliver significant protection of brain in animal model of stroke**
- **Data presented today to international conference**
- **Clears way for NYX-104 to be developed as neuroprotectant.**

Sydney, 29 November 2017: Noxopharm and its US subsidiary, Nyrada Inc, are pleased to announce jointly that the experimental drug, NYX-104, has cleared a critical step in its development as a neuroprotective drug.

Data released today at the 12th Cerebral Vascular Biology International Conference being held in Melbourne shows that NYX-104 was able to provide a significant level of neuroprotection in a mouse model of human stroke.

Any injury to the brain (e.g. stroke, concussion, head trauma, severe epilepsy, concussive noise) is associated with 2 phases of brain cell death. The first phase is immediate and involves the death of brain cells directly injured by the trauma. The second phase extends over days and weeks and involves a wave of cell death that radiates out from the original site of injury, potentially resulting finally in an area of brain death many times greater than the original injury. This second phase is known as ***excitotoxicity***. The extent of excitotoxicity can mean the difference between making a full recovery from stroke with minimal rehabilitation, to being left months later with significant functional impairment despite lengthy rehabilitation.

Excitotoxicity is a major public health issue, and finding an effective drug therapy that can be administered following stroke or head trauma has been identified as an important and urgent medical need. Recent publicity around sporting concussion injury (egg. football and boxing) has brought the matter into the public domain.

Noxopharm announced recently (3 November 2017) that a collaboration between NOX and the University of NSW (Sydney) had identified NYX-104 as a potential drug candidate to treat excitotoxicity. That report was based on an ability to inhibit the excitotoxicity reaction *in vitro*. The data reported on today confirms that this inhibitory effect carried through *in vivo*, confirming the

ability of the drug to cross the blood-brain barrier, and clearing the way for Nyrada to bring NYX-104 through into the clinic.

Noxopharm and Nyrada CEO, Dr Graham Kelly, said," The data released today means that Nyrada can now proceed with confidence to develop NYX-104 as a neuroprotective drug for the treatment for excitotoxicity. We will be focusing in the first instance on using the drug to protect the brain from stroke injury. NYX-104 cannot stop stroke from occurring, but by administering the drug once stroke has occurred, the aim will be to limit the extent of the second wave of brain damage. Less damage should mean less loss of function, shorter recovery periods, and greater likelihood of making a full recovery."

"And given the potential role of excitotoxicity in anything that kills brain cells ranging from physical trauma through to degenerative disease (Alzheimer's, Parkinson's etc.), a successful neuroprotectant has significant community use. Nyrada has an aim of bringing NYX-104 into a human trial in 2019," Kelly added.

The presentation can be found on the Nyrada website (www.nyrad.coom)

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About Excitotoxicity

Excitotoxicity refers to the process where healthy neurons (nerve cells) are killed largely as a result of the mobilisation of stores of calcium ions in the cell. The calcium mobilisation is triggered by an outpouring of glutamate from damaged neurons, with toxic levels of calcium activating a number of enzymes within the receiving neuron leading to its death. Excitotoxicity is a cascading process of death of neurons following an original focus of damage and is a major contributor to limited recovery following initial brain injury. Excitotoxicity features in stroke, traumatic brain injury, epileptic seizure, spinal cord injury and likely contributes to neurodegenerative diseases of the central nervous system such as multiple sclerosis, Alzheimer's Disease, Huntington's Disease, Parkinson's Disease and amyotrophic lateral sclerosis (ALS).

About NYX-104

NYX-104 is a small molecule kinase-inhibitor that blocks TRPC class ion channel-regulated influx of calcium ions and mobilisation of calcium stores in axons exposed to glutamate overload.

About Noxopharm

Noxopharm is an Australian drug development company with offices in Sydney and Hong Kong. The Company has a primary focus on the development of drugs to sensitise cancer cells to radiotherapy. NOX66 is the first pipeline product, with later generation drug candidates under development.

About Nyrada Inc.

Nyrada Inc is a US biotechnology company, established as a subsidiary of Noxopharm to focus on non-oncology drug development. Nyrada has 3 drug assets: NYX-104 (excitotoxicity inhibitor), NYX-205 (anti-inflammatory), NYX-330 (PCSK9 inhibitor).

Investor & Corporate Enquiries:
Prue Kelly
M: 0459 022 445
E: info@noxopharm.com

Company Secretary:
David Franks
T: +61 2 9299 9690
E: dfranks@fa.com.au

www.noxopharm.com

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Identification of NYX-104, a promising lead candidate for the treatment of stroke and other excitotoxicity-associated disorders

Nagarajesh Gorlamandala[1], Jasneet Parmar[1], Chamini J. Perera[1], Georg von Jonquieres[1], Benny J. Evison[2], Graham E. Kelly[2] and Gary D. Housley[1]

[1]Translational Neuroscience Facility and Department of Physiology, School of Medical Sciences, UNSW Sydney, NSW 2052, Australia
[2]Nyrada Inc., Sydney, NSW, Australia

ABSTRACT

Introduction. Excitotoxicity is a feature of many CNS-based disorders and is characterised by neuron death following sustained stimulation by neurotransmitters such as glutamate. We screened a range of isoflavones for neuroprotection against metabotropic glutamate receptor – driven neuronal Ca^{2+} influx arising from TRPC cation channel activation through the Gq–phospholipase C-PIP_2–diacylglycerol pathway. This is blocked by the isoflavone genistein, however the compound demonstrates unfavourable pharmacokinetic properties.
Aims. To identify compounds with more desirable features, a library of isoflavones was screened for their capacity to block Ca^{2+} influx and confer neuroprotection.
Methods. HEK293 cells stably expressing GCaMP5G, a genetically encoded Ca^{2+} reporter, were exposed to proprietary isoflavones and Ca^{2+} influx was measured following stimulation by carbachol, an agonist of endogenous Gq–type muscarinic receptors. Using a FlexStation 3 multimodal plate reader, Ca^{2+} store release and Ca^{2+} entry via endogenous genistein-sensitive TRPC-like channels was quantitated. Selected isoflavones were then evaluated for neuroprotection using a mouse (C57Bl/6J strain) photothrombotic stroke model whereby isoflavones were administered rectally (100 mg/kg/d isoflavone or vehicle alone) once daily for five days. Brain tissue was then imaged for infarct size.
Results. Among the isoflavones assayed in vitro, NYX-104 was most proficient, impairing both Ca^{2+} store release and extracellular Ca^{2+} re-entry equipotently (IC_{50} ~ 700 nM). In vivo, mice receiving NYX-104 demonstrated a striking decrease in cerebral cortex infarct penumbra expansion of ~ 50% in surface area relative to vehicle-treated controls. NX-101, an analogue that exhibited modest activity in vitro (IC_{50} ~ 1.5 μM), failed to reduce lesion area.
Discussion. A promising lead candidate for stroke therapy and potentially other excitotoxicity-associated disorders has been identified. NYX-104 inhibits both Ca^{2+} store release and Ca^{2+} re-entry mechanisms equipotently, perhaps reflecting a common upstream target. Proof-of-concept has been achieved in vivo with NYX-104 but not NX-101, suggesting NYX-104 has unique properties not necessarily shared amongst isoflavones.

INTRODUCTION

- Stroke is one of the nation's single largest killers and is a leading cause of disability [1].
- The condition is associated with excitotoxicity; an excessive stimulation of neurons by the neurotransmitter glutamate [2].
- Glutamate exposure initiates Ca^{2+} neuronal influx through TRPC cation channels following activation of metabotropic glutamate receptors (Figure 1) [3].

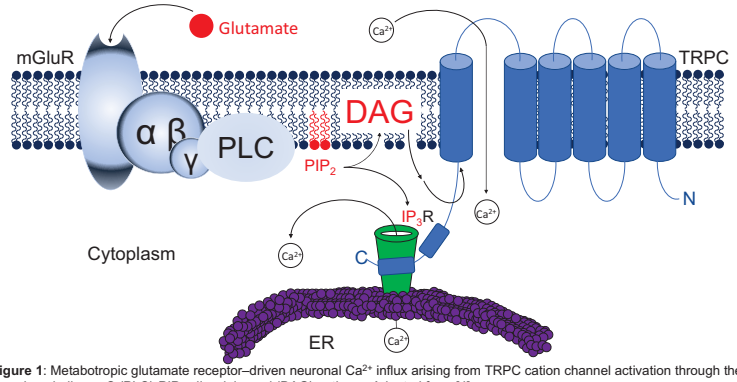


Figure 1: Metabotropic glutamate receptor–driven neuronal Ca^{2+} influx arising from TRPC cation channel activation through the Gq–phospholipase C (PLC)-PIP_2–diacylglycerol (DAG) pathway. Adapted from [4].

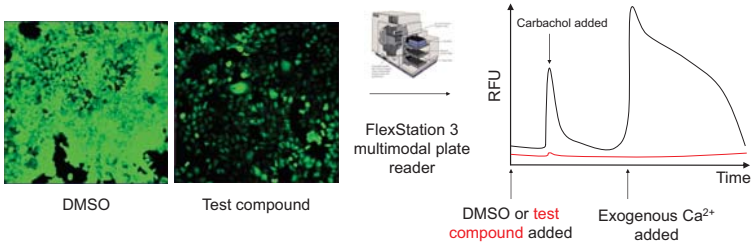
- Ca^{2+} influx culminates in neuronal death. Left untreated, this process is amplified in a chain reaction of cell death [2].

AIMS

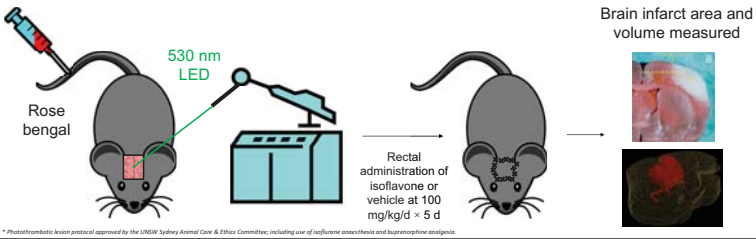
- To identify small molecule inhibitors of the Gq-PLC-PIP_2-DAG pathway that block Ca^{2+} influx and confer neuroprotection.

METHODS

In vitro. rHEK293 cells stably expressing a GCaMP5G Ca^{2+} reporter.



In vivo. Photothrombotic ischemia C57Bl/6J mouse model*.



* Photothrombotic lesion protocol approved by the UNSW Sydney Animal Care & Ethics Committee; including use of isoflurane anaesthesia and buprenorphine analgesia.

RESULTS

In vitro.

- All isoflavones inhibited both Ca^{2+} endoplasmic reticulum store release and extracellular Ca^{2+} influx (e.g. NYX-104, Figure 2).
- NYX-104 was most active, inhibiting both Ca^{2+} mobilisation pathways equipotently (Table 1).

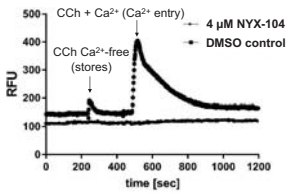


Figure 2: A representative trace (solid circles, DMSO control) demonstrating the fluorescence intensity of the cytoplasmic GCaMP5G Ca^{2+} reporter as a function of time in HEK293 cells. Ca^{2+} from internal stores was purged from the ER via the addition of 100 μM carbachol (CCh in Ca^{2+}-free media) to initiate signalling through the Gq-PLC-PIP_2-DAG pathway (Figure 1). Following the return of Ca^{2+} to the media, a prominent increase in fluorescence reflects cellular Ca^{2+} influx via Ca^{2+} entry pathways (TRPC-like channels). On the addition of 4 μM NYX-104 (solid squares, lower trace), Ca^{2+} mobilisation through both pathways is completely suppressed. Flexstation RFU – relative fluorescence units (Ca^{2+} signal).

Pathway	Genistein	NX-101	NYX-104	NX-103	NX-104	NX-105	NX-106
Ca^{2+} influx	4	1.5	0.7	>> 0.7	>> 0.7	>> 0.7	0.9
Ca^{2+} store release	4	1.5	0.7	>> 0.7	>> 0.7	>> 0.7	0.7

Table 1: The concentration of each isoflavone where either Ca^{2+} influx or Ca^{2+} store release is inhibited by half (IC_{50}).

- The library of isoflavones demonstrated a spectrum of activities, ranging from mildly active to potent (Table 1).
- NX-106 was unique in exhibiting different IC_{50} values for Ca^{2+} influx and Ca^{2+} store release pathways.
- *In vivo*.
- NYX-104 conferred significant protection against the expansion of the infarct penumbra in the context of lesion area (Figure 3A – E) and volume (Figure 3F).
- In parallel, NX-101 failed to confer neuroprotection.

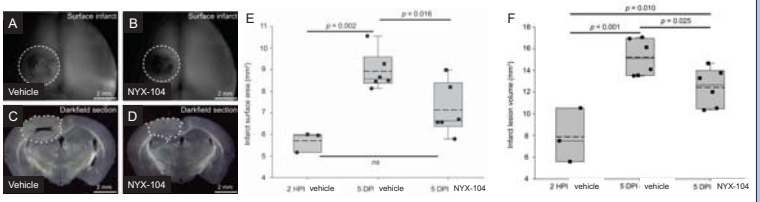


Figure 3: (**A** – **D**) Representative images of infarct lesions (delineated by white dotted boundaries) on the cerebral cortex surface (**A**, **B**) or within a coronal cryosection (50 μm) 5 days post injury in mice treated with either vehicle (**A**, **C**) or NYX-104 (**B**, **D**). (**E**, **F**) Box plots comparing cerebral cortex lesion area (**E**) and volume (**F**) either 2 h post injury (2 HPI) or 5 days post injury (5 DPI). Data are overlaid; box plots fills show 25% and 75% data boundaries with bars showing 95% boundaries.

DISCUSSION

- Extracellular Ca^{2+} influx and Ca^{2+} store release are spatiotemporally distinct pathways (Figure 1). The equipotent nature of NYX-104-induced impairment may reflect a common target upstream of both release pathways.
- *In vitro*, this notion holds true for all isoflavones with a clear exception in NX-106.
- Structure-activity-relationships have emerged from screening the library, guiding the design of next-generation inhibitors.
- Proof-of-concept has been achieved *in vivo* with NYX-104 but not NX-101, indicating NYX-104 has unique properties not shared by all isoflavones.

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