Date: 15th December 2020
ASX Announcement (ASX: IHL)

Positive *in vivo* results confirm strong synergistic neuroprotective activity of IHL-216A after traumatic brain injury

***In vivo* examination confirms that IHL-216A exhibits stronger neuroprotective properties than CBD alone; favourable international search report and opinion received on the International Patent Application**

Highlights:

- IHL-216A components, cannabidiol ('CBD') and isoflurane, act synergistically to reduce neuronal damage, neuroinflammation and behavioural deficits that are consequences of traumatic brain injury ('TBI')
- Key results:
    - IHL-216A outperformed CBD in reducing neuronal damage in post-mortem Nissl staining analysis of brain tissue by 53% for CA1 and 60% for CA2 in the hippocampal region of the brain
    - IHL-216A reduced the Iba1 neuroinflammation marker by 35% more than CBD alone and 123% more than isoflurane administered alone
    - IHL-216A outperformed CBD and isoflurane in animal behavioural tests
- An International Patent Application entitled "Compositions and methods for the treatment or prevention of traumatic brain injury" was recently filed as part of the IHL-216A development program
- The International Search Report and Opinion has also been received on the recently filed International Patent Application, with the International Examiner indicating that claims directed to IHL-216A and methods for the treatment of TBI using IHL-216A are novel, inventive and satisfy the industrial applicability requirements
- TBI accounts for approximately 10 million deaths and/or hospitalisations annually in the world (Schuman et al., 2017) and there are currently no registered pharmaceutical agents approved for the treatment of TBI.

Clinical stage pharmaceutical development company, Incannex Healthcare Limited (ASX: IHL, 'Incannex' or the 'Company'), is pleased to announce that it has received positive results from Creative Biolabs (USA) in relation to the neuroprotective capability of IHL-216A in preclinical *in vivo* (animal) studies.

IHL-216A is a combination drug that combines cannabidiol ('CBD') with any volatile anaesthetic agent, including isoflurane ('isoflurane'), which has been used in this study. IHL-216A has been designed to be administered soon after head trauma to reduce secondary brain injuries that lead to neurological deficits. Secondary brain injuries evolve over minutes, days and months after the primary insult and result from

biochemical, metabolic, and cellular changes initiated by the primary event. Due to the product's potential therapeutic utility in contact sports, IHL-216A is designed to satisfy World Antidoping Authority (WADA) and Australian Anti-Doping Authority's (ASADA) specifications for use by athletes at risk of TBI and Chronic Traumatic Encephalopathy, otherwise known as CTE.

Overview

To investigate whether CBD and isoflurane have synergistic neuroprotective effects, Incannex employed a rat controlled cortical impact (CCI) model of traumatic brain injury. The injury was induced in the rats by first performing a craniotomy to remove a small section of bone from the skull and then the injury is induced using a pneumatic rod to directly impact the exposed brain.

The animals were treated with CBD and isoflurane both alone and in combination using the same doses. Isoflurane was administered by inhalation for 30 min, 1-2 hours after injury, only on the day of injury. CBD was administered by intraperitoneal injection every day for 7 days post injury with the first dose administered 15-30 minutes post injury. A vehicle treated control group and sham-injured (uninjured) group where the craniotomy, but not injury, was performed, were also included. The study involved a total of 108 rats across the treatment groups.

Damage caused in traumatic brain injuries has both molecular and behavioural consequences. Thus, four distinct analytical techniques were implemented to determine whether the CBD and isoflurane (IHL-216A) combination benefitted animals subjected to TBI and whether the combination was synergistic. These techniques included Nissl staining and the measurement of neuroinflammation marker Iba1 to assess the effect of CBD and isoflurane at the cellular and molecular levels. The Morris Water Maze and the Rotarod Performance Test were used as behavioural tests.

Results

Nissl Staining of Brain Tissue

Post-mortem analysis of rat brains also detected synergy between CBD and isoflurane (IHL-216A). Brains were fixed and sectioned prior to Nissl staining to identify neuronal damage (1, 2). Nissl staining is a process where very thin sections of brain tissue are stained with a dye that binds to specific components of the cell called Nissl bodies which can then be viewed under a microscope. Healthy neurons typically have more Nissl bodies than damaged ones. Neuronal damage is indicated by the ratio of Nissl bodies to neurons across different sections of the hippocampus with a lower Nissl/neuron ratio indicative of increased neuronal damage. Synergy between CBD and isoflurane (IHL-216A) was detected in hippocampal regions *cornu ammonis* 1 (CA1) and CA2 (Figure 1, Appendix 1). These regions of the brain are known to be important in the formation and storage of memories (3, 4). In the experiment, IHL-216A outperformed CBD alone by 53% for CA1 and 60% for CA2, demonstrating less neuronal damage experienced by the rats relative to CBD.

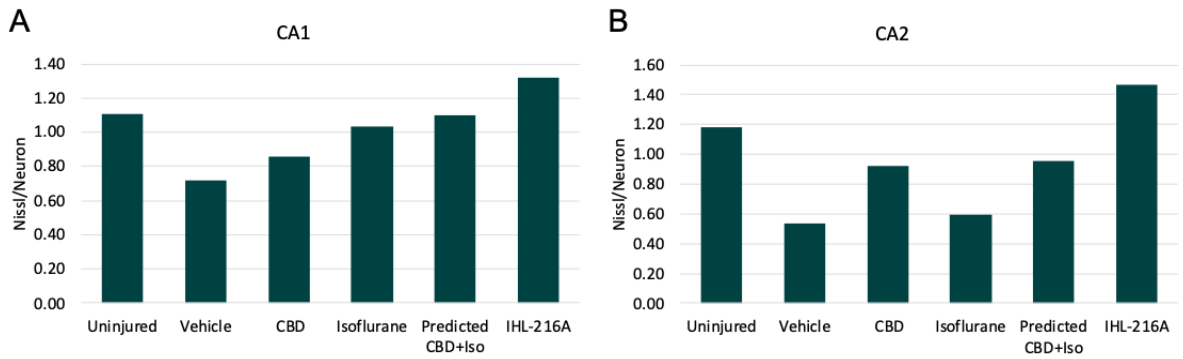

Figure 1. Synergistic activity of CBD and isoflurane (IHL-216A) in neuronal damage as assessed by Nissl staining. Rodents that had undergone TBI and treated with CBD and/or isoflurane or vehicle were assessed for neuronal damage by post-mortem analysis of fixed brain sections by Nissl staining. Nissl staining permits the quantitation of the ratio of Nissl bodies to total neurons, a lower ratio being indicative of increased neuronal damage. The Nissl/neuron ratio observed in hippocampal regions (A) CA1 and (B) CA2 contralateral to the site of injury in the group treated with CBD and isoflurane (IHL-216A) was greater than that predicted based on the groups treated with each drug alone, which is indicative of synergy. Values are average of the respective treatment group. Group sizes: Uninjured n=6, vehicle n=6, CBD n=6, isoflurane n=5, IHL-216A n=6.

Neuroinflammation Marker - Iba1

The activity of CBD and isoflurane (IHL-216A) was also synergistic in reducing levels of the neuroinflammation marker Iba1 (5) as detected using immunofluorescence (Figure 2, Appendix 1). Iba1 is a protein expressed in microglia, a type of innate immune cell in the brain, that is an established marker of microglial activation and neuroinflammation. The levels of Iba1 in the brain are detected using immunofluorescence, which is a microscopy technique that employs antibodies specific to Iba1 which are detected through the use of a fluorescent tag. Increased levels of Iba1 are indicative of increased neuroinflammation. IHL-216A reduced the Iba1 neuroinflammation marker by 35% more than CBD alone and 123% more than isoflurane administered alone.

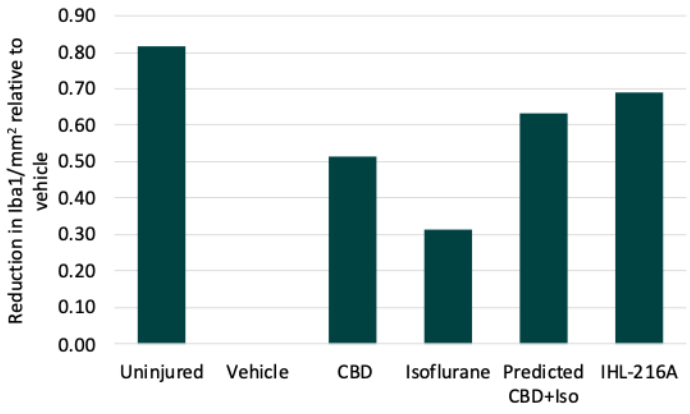

Figure 2. Synergistic activity of CBD and isoflurane (IHL-216A) in reducing levels of the neuroinflammatory marker Iba1. Rodents that had undergone TBI and treated with CBD and/or isoflurane or vehicle were assessed for neuroinflammation through immunofluorescence analysis of the neuroinflammatory marker Iba1. Iba1 levels increase after TBI and a reduction in Iba1 is indicative of a reduction in neuroinflammation. Iba1 levels in brain sections ipsilateral to the site of injury in the group treated with CBD and isoflurane (IHL-216A) were reduced more than would be predicted based on the reduction observed in groups treated with each drug alone, which is indicative of synergy. Values are average of the respective treatment group. Group sizes: Uninjured n=6, vehicle n=5, CBD n=6, isoflurane n=3, IHL-216A n=5.

Morris Water Maze

Synergy between CBD and isoflurane (IHL-216A) was detected in the behavioural outcomes assessed using the Morris Water Maze (Figure 3, Appendix 1) and Rotarod Performance Test (Figure 4, Appendix 1). In the Morris Water Maze animals are trained to find a platform in a pool of water. After a number of training sessions, the platform is removed and the mice are monitored to determine whether they return to the location of the platform, which is a measure of spatial learning and memory (6, 7). The synergistic effect of CBD and isoflurane (IHL-216A) was observed. IHL-216A outperformed CBD alone when assessing both the number of times rats returned to the location of the platform per group by 275% as well as the proportion of rats in the group that returned to the location of the platform by 67%.

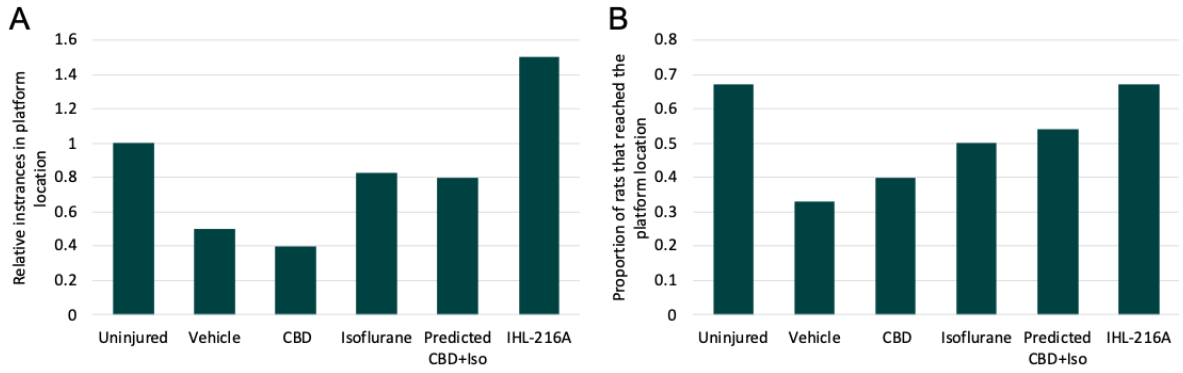

Figure 3. Synergistic activity of CBD and isoflurane (IHL-216A) in the Morris Water Maze assessment. Rodents that had undergone TBI and treated with CBD and/or isoflurane or vehicle were assessed for spatial learning and memory using the Morris Water Maze. The observed performance with respect to both (A) relative instances of animal in platform location and (B) proportion of animals in that reached the platform location was better in the group treated with the CBD isoflurane combination (IHL-216A) than what was predicted based on the performance of the groups treated with each drug alone. This outperformance by the IHL-216A compared to the predicted performance is indicative of synergy. Values are average of the respective treatment group. Group sizes: Uninjured n=6, vehicle n=6, CBD n=5, isoflurane n=6, IHL-216A n=6.

Rotarod Performance Test

In the Rotarod assay, rats are placed on a rotating cylinder and monitored for the time it takes them to fall off, or latency (8, 9). A shorter latency is indicative of a motor function deficit. IHL-216A was also synergistic

in increasing Rotarod latency in animals with TBI, exceeding CBD alone by 10% and isoflurane by 12% in the experiment.

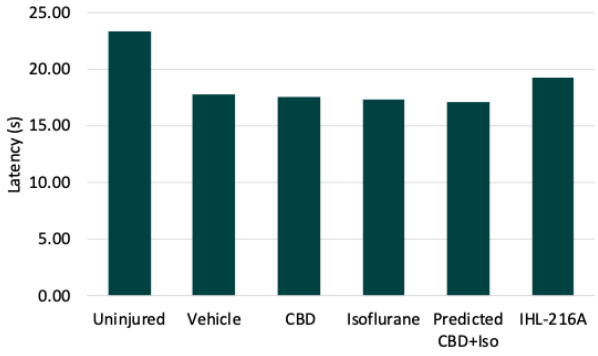

Figure 4. Synergistic activity of CBD and isoflurane (IHL-216A) in the Rotarod assessment. Rodents that had undergone TBI and treated with CBD and/or isoflurane or vehicle were assessed for motor function using the Rotarod. The observed latency in the group treated with the combination of CBD and isoflurane (IHL-216A) was greater than that predicted based on the groups treated with each drug alone. The improved performance of in the group treated with the combination compared to that predicted based on the performance of the groups treated with each drug alone is indicative of synergy. Values are average of the respective treatment group. Group sizes: Uninjured n=6, vehicle n=6, CBD n=5, isoflurane n=6, IHL-216A n=6.

Intellectual Property Considerations

An International Patent Application entitled "Compositions and methods for the treatment or prevention of traumatic brain injury" (Authorised Officer Tracey Huynh, AUSTRALIAN PATENT OFFICE, International application no. PCT/AU2020/051056) was recently filed as part of the IHL-216A development program. This application was filed pursuant to the Patent Cooperation Treaty (PCT), thus providing IHL with an opportunity to pursue patent protection in foreign jurisdictions, including key markets with established and developing medicinal cannabis industries.

The PCT Application process includes the conduct of preliminary searches by the International Searching Authority to identify prior art that may be relevant to the novelty and inventive step of the claims as filed. The results of such preliminary searches are published in the form of an International Search Report and Opinion, which is followed by the International Preliminary Report on Patentability.

The International Search Report and Opinion of the International Searching Authority have now been received by IHL. Pleasingly, the International Examiner considers that claims directed to the IHL-216A and methods for the treatment of TBI using IHL-216A are novel, inventive and meet the requirements for industrial applicability. Based on the International Search Report and Opinion, IHL is currently considering options to expedite the filing and examination of patent applications in key jurisdictions as part of IHL's intellectual property (IP) strategy.

CEO and Managing Director of Incannex Healthcare, Mr Joel Latham said; "The results of the animal study are extremely pleasing and steadfastly demonstrate significant improvements in the key markers of secondary brain injury in a highly-controlled environment. Our intent is for IHL-216A to be the first line of defence to dampen the short and long-term effects of traumatic brain injury caused by any means, but particularly in contact sports, such as the NFL and the major contact sporting codes in Australia where CTE is a growing concern".

Incannex is currently assessing the most efficient clinical trial program to follow the successful *in vivo* studies so that it may pursue an FDA new drug application, subject to further clinical success, as quickly as possible. The Company anticipates that clinical program will be established in early 2021.

About Traumatic Brain Injury and Concussion

TBI accounts for approximately 10 million deaths and/or hospitalization annually in the world (Schuman et al., 2017). There are currently no registered pharmaceutical agents approved for the treatment of TBI. Current treatment of major TBI is primarily managed through surgical intervention by decompressive craniotomy (Bullock et al., 2006) which involves the removal of skull segments to reduce intracranial pressure.

ENDS

The release of this announcement has been approved for issue by IHL's Board of Directors. For further details on the announcement, interested parties should contact:

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About Incannex Healthcare Limited (ASX: IHL)

Incannex Healthcare Limited (IHL.ASX) is a clinical stage pharmaceutical development company developing unique medicinal cannabis pharmaceutical products and psychedelic medicine therapies for the treatment of Generalised Anxiety Disorder (GAD), Obstructive Sleep Apnoea (OSA), Traumatic Brain Injury (TBI)/Concussion and Acute Respiratory Distress Syndrome (ARDS). FDA registration, subject to ongoing clinical success, is being pursued for each product and therapy under development.

Each indication represents major global markets and currently have no, or limited, existing registered pharmacotherapy (drug) treatments available to the public, raising the possibility of patients receiving Government subsidies for products that demonstrate suitable safety and efficacy profiles in clinical trials.

IHL has a strong patent filing strategy (as announced "IHL files cannabinoid patent over IHL-216A for TBI" 04th October, 2019 and "IHL Files Patent over IHL-42X for OSA" 06th of December, 2019) as it develops its products and therapies in conjunction with its medical advisory board.

Further to its clinical programs, Incannex has its Australian license to import, export and distribute medicinal cannabis products and has launched a line of cannabinoid oil products. The cannabis-based oils are sold under Incannex's product supply and distribution agreement with Cannvalate Pty Ltd, which is the largest network of cannabis medicine prescribers in Australia and a major shareholder of Incannex.

Website: www.incannex.com.au

Investors: investors@incannex.com.au

Appendix 1

The predicted combinatorial effects of the drugs were calculated using the Bliss independence principle using the equation: $E_{pred\ A+B}=(E_A+E_B)-(E_AE_B)$, where E_A is the activity of drug A on its own and E_B is the activity of drug B on its own. Synergy between the two drugs is said to occur when the observed activity of the drugs in combination is greater than predicted activity.

Table 1. Values used to determine synergy between CBD and isoflurane

	Relative instances in platform location	Proportion of rats that reached the platform location	Rotarod Latency (s)	Nissl/Neuron CA1	Nissl/Neuron CA2	Reduction in Iba1 count/mm² relative to vehicle
Uninjured	1.00	0.67	23.39	1.10	1.18	0.82
Vehicle	0.50	0.33	17.83	0.72	0.54	0.00
CBD	0.40	0.40	17.60	0.86	0.92	0.51
Isoflurane (ISO)	0.83	0.50	17.33	1.03	0.60	0.31
Predicted CBD+ISO	0.80	0.54	17.08	1.10	0.96	0.63
IHL-216A	1.50	0.67	19.33	1.32	1.47	0.69
EOB	0.70	0.13	2.25	0.22	0.51	0.06
% Outperform CBD	275%	67%	10%	53%	60%	35%
% Outperform ISO	81%	34%	12%	28%	145%	123%
% outperform Pred	87%	24%	13%	20%	53%	10%

Calculation definitions for Table 1:

- EOB = IHL-216A – Predicted CBD+ISO
- % Outperform CBD = (IHL-216A/CBD) - 1
- % Outperform ISO = (IHL-216A/ISO) – 1
- % Outperform Pred = (IHL-216A/Predicted CBD+ISO) - 1