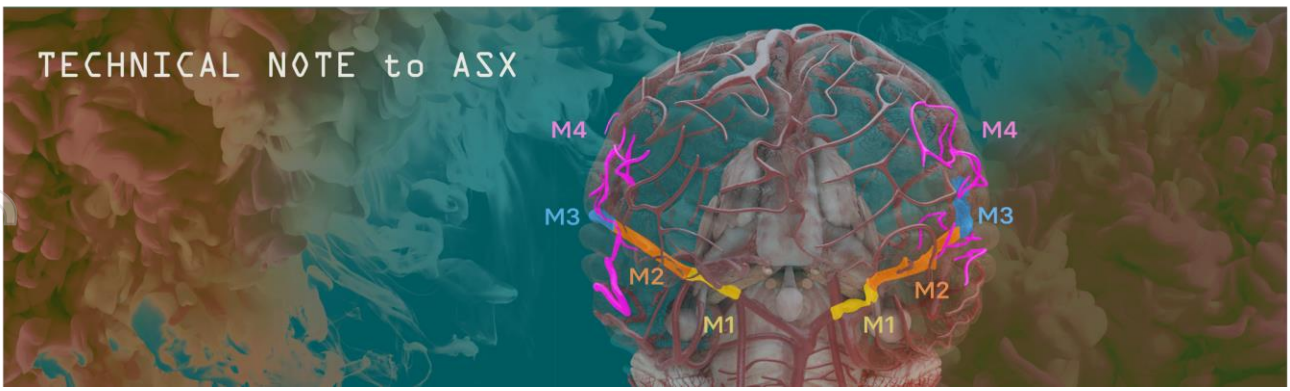
# Arterial ischaemic stroke (AIS)

CLINUVEL

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| **Melbourne, Australia, 20 March 2023** | **ASX:** | **CUV** |
| | **Börse Frankfurt:** | **UR9** |
| | **ADR Level 1:** | **CLVLY** |

CLINUVEL today announced that the first arterial ischaemic stroke (AIS) patient received treatment with PRÉNUMBRA® Instant as part of the CUV803 study, the Company's second study of afamelanotide in AIS. This Technical Note provides further detail on the current standards of care in stroke, the potential of melanocortins to treat stroke patients, and the aims of CLINUVEL's stroke program.

## The CUV803 study

The phase II CUV803 study aims to investigate the dose response and safety of PRÉNUMBRA® Instant (afamelanotide) in acute AIS. The study design and protocol take key learnings from the clinical data generated in the first stroke study, CUV801, which showed that afamelanotide (16mg implant) was well tolerated by patients in a uniform dose, with the majority of patients showing a functional recovery post-treatment. The CUV803 study will provide further information on the safety and clinical benefits of afamelanotide at a higher, and more frequent, dosing regimen using a fast-acting liquid formulation.

In line with the aims of CLINUVEL's stroke program, CUV803 will assess the impact of PRÉNUMBRA® Instant treatment on the ischaemic core and penumbra and will also investigate the effect on cerebral fluid based on afamelanotide's anti-oncotic and anti-oxidative properties.

## Clinical evaluations

**In evaluating PRÉNUMBRA® Instant in AIS patients, various clinical assessments will be made throughout the CUV803 study:**

**Safety** – Monitoring and recording treatment-emergent adverse events and concomitant medications throughout the course of the study.

**National Institutes of Health Stroke Scale** – The validated NIHSS records the level of impairment caused by a stroke in 11 items, giving an overall assessment score between 0 (no impact) and 42 (death).

**Activities of Daily Living** – The ADL indicates the degree of disability or dependence in the daily activities of patients who have suffered a stroke or other causes of neurological disability.

**Cognition** (Mini Mental State Examination, MMSE) – A 30-point cognitive assessment commonly used to assess the level of cognitive impairment following a stroke.

**Imaging** – Patients will be evaluated through computed tomography perfusion (CTP) of the cerebrum, which will be compared to Magnetic Resonance Imaging (MRI), Diffusion Weighted Imaging (DWI) and Fluid Attenuated Inversion Recovery (FLAIR).

For patients with acute arterial occlusion, computed tomography perfusion relative cerebral blood flow (CT-rCBF) maps provide a fast and accurate estimate of tissue that is likely to be irreversibly injured in acute stroke patients.

## What is a stroke and what happens following an arterial ischaemic stroke (AIS)?

Following the occlusion of a blood vessel in the brain by a clot (stroke), several events contribute to immediate and irreversible brain damage. This results in tissue being deprived of oxygen and other nutrients swiftly leading to progressive cell death. Immediately, a central "core" of necrotic cells is formed, surrounded by a peripheral area of tissue at risk of dying off (the penumbra) over an extended period of time.

Stroke causes a disruption of the blood brain barrier (BBB), which allows transport of ions and substances directly to the brain, contributing to fluid formation. In the acute phase of a stroke, immune cells (neutrophils and macrophages) are accumulated, contributing to the progression of brain injury. Macrophages and activated microglia release pro-inflammatory cytokines (tumour necrosis factor (TNF-α), interleukin (IL)1-β, IL-1, IL-6), enhancing the expression of intercellular adhesion molecule-1 (ICAM-1), P-selectin, and E-selectin, and enabling immune cells to bind to the vessel wall and transmigrate into tissues.

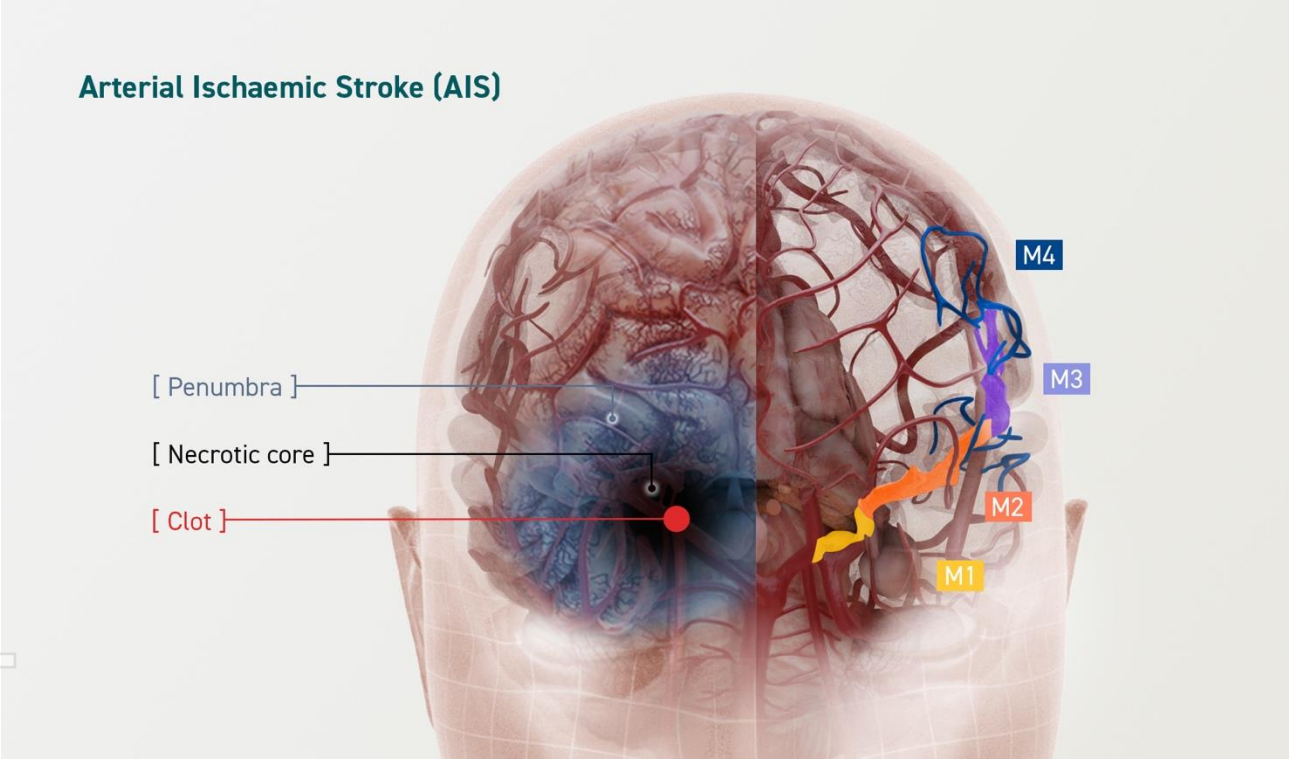


***Figure 1*** *A clot lodged in a brain vessel causes a central zone of dead brain tissue, known as the necrotic core. The area surrounding the core, called the penumbra, is at immediate risk of tissue death: this brain tissue can be returned to normal function if immediate intervention with a drug or clot removal can be offered. The right side of the image shows the middle cerebral artery (MCA), branches from M1–M4.*

## AIS treatment

Stroke is a leading cause of death and disability in the world. In some cases, intravenous thrombolysis (clot-bursting drugs; IVT) or mechanical thrombectomy (physical removal of the clot; EVT) may be used to restore blood flow to the brain, often saving the life of a patient or severely reducing the overall impact of brain damage.

IVT involves the injection of tissue plasminogen activator (tPA), such as alteplase, into the affected artery to dissolve the blood clot. The medication breaks up the clot, allowing blood to flow to the brain. IVT use varies from country to country, but in general it is recommended in patients with acute ischaemic stroke within 4.5 hours of admission to a hospital, or less than 9 hours when brain imaging shows "perfusion mismatch" and where mechanical thrombectomy is either not indicated or planned. This critical therapeutic window makes IVT severely limited in its application, particularly when the time of onset of the stroke is not known. Thus, alteplase is only given to <20% of patients worldwide.

Likewise, recommendations for EVT vary from country to country, but this intervention is generally recommended within six hours after start of symptoms. EVT is also greatly limited by the location of the blocked artery in a patient's brain, with those clots in the upper regions – so-called "M2" and above – largely unretrievable through thrombectomy.

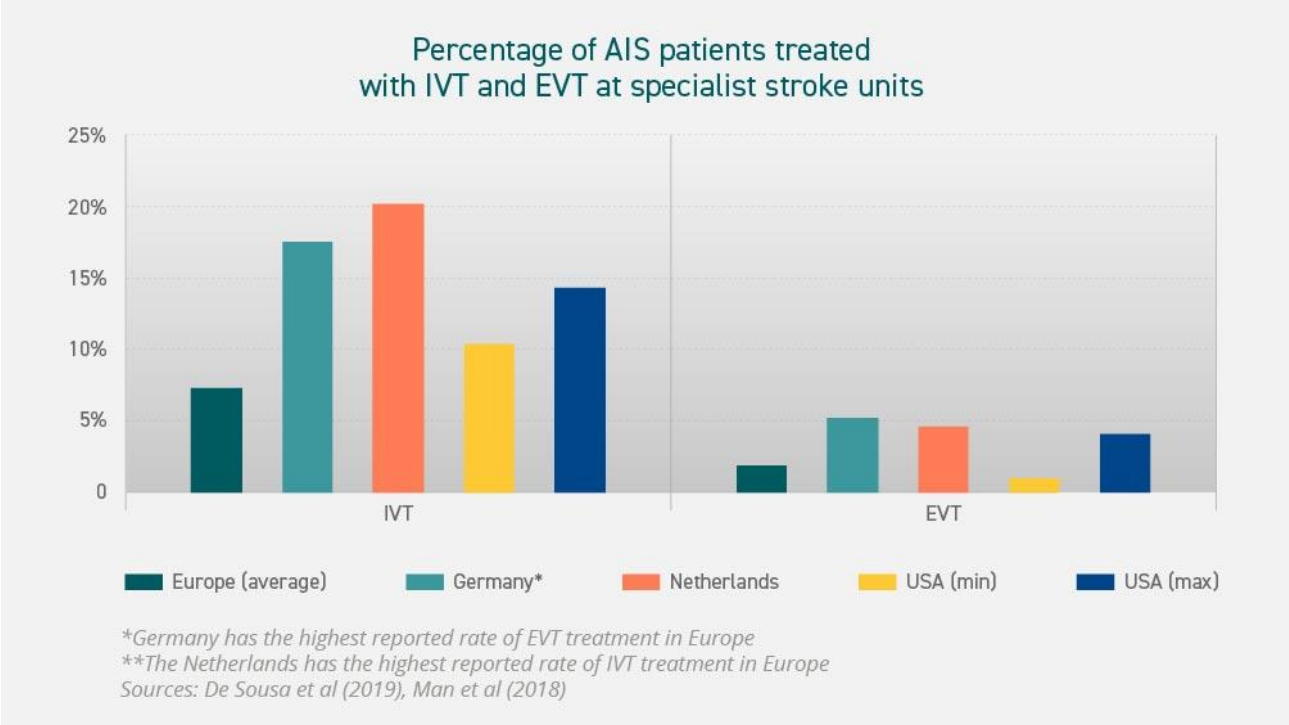


***Figure 2*** *Percentage of AIS patients treated with EVT and IVT at specialist stroke units.*

## Melanocortins in AIS

There is a clear need for novel stroke treatments, either as a single drug or in combination with the other two modalities. The safety data collected over more than two decades suggest afamelanotide may be well tolerated, while there is limited scope for drug-drug interactions.

The neuroprotective, anti-oxidative, anti-oncotic and anti-inflammatory characteristics of melanocortins (MCs), including afamelanotide, have been a focus for researchers. As melanocortin receptors (MCRs) are expressed in the cerebral endothelium, there is strong justification for further exploration of their potential role in stroke and central nervous system (CNS) disorders.

Melanocortins have the potential to be used as a therapeutic treatment for AIS due to their **neuroprotective properties**, as demonstrated in preclinical studies. The effects occur through antagonism of excitotoxic, inflammatory, and apoptotic responses, and the drug might also promote functional recovery through stimulation of repair mechanisms, including neurogenesis, as discussed in further detail below. In detail, afamelanotide may assist in stroke by:

I. Supressing the exitotoxic reaction in the brain and peripheral tissues as well as the inflammatory and apoptotic cascades. This reduces BBB disruption and prevents oedema formation.

II. Activating the α-MSH/MCR signalling pathways which possess potent anti-inflammatory properties, inhibiting the production of pro-inflammatory cytokines and promoting the release of anti-inflammatory cytokines. This anti-inflammatory effect is mediated by the activation of the cAMP/PKA pathway, which leads to the inhibition of NF-κB signaling and the subsequent downregulation of inflammatory gene expression. Through this mechanism of action, afamelanotide has the potential to reduce the expression and release of these cytokines in stroke, leading to reduced brain injury and improved neurological outcomes. Targeting inflammation and apoptosis may rescue part of the viable brain tissue in the penumbra, thus reducing overall brain damage.

III. Exerting vasodilatory properties. MCs increase vascular relaxation upregulating signalling of the NO-cGMP-pathway There is further potential to increase blood flow to the tissue by affecting the local control of vascular tone, improving endothelial function augmenting NO availability, activating endothelial NO synthase (eNOS) through endothelial melanocortin 1 receptors (MC1Rs) and reducing ROS mediated breakdown of NO, enhancing endothelium-dependent vasodilatation. NO diffuses from the endothelium to the vascular smooth muscle, provoking muscle relaxation, inhibiting excessive cell proliferation (inducing cell cycle arrest at low levels) and preventing endothelial apoptosis and leukocyte adhesion, thus regulating vascular health. NO has anti-adhesion and anti-inflammatory properties as it downregulates endothelial adhesion molecule expression and inhibits platelet aggregation in low levels. The resulting vasodilative effect has the potential to reduce the ischaemic tissue damage, and aid in the salvaging of the ischaemic penumbral area.

IV. Increasing the expression of manganese-dependent superoxide dismutase (Mn-SOD) expression, a ROS scavenger, through the activation of endothelial MC1Rs. Mn-SOD inhibits ROS such as superoxide ion (02-), which can reduce NO bioavailability, preventing NO from diffusing to its target sites and causing injury to the endothelium. 02- can react with NO to form peroxynitrite (ONOO-), which can lead to the reduction of NO production. α-MSH reduces the induction of iNOS and COX-2 gene expression at the hypothalamic level during endotoxemia and suggest that endogenous α-MSH may exert an inhibitory tone on iNOS and COX-2 transcription via MC4R acting as a local anti-inflammatory agent within the hypothalamus.

    Afamelanotide has the potential to upregulate signalling of the nitric oxide-cGMP pathway by activating eNOS (mediated by endothelial MC1Rs), thus promoting endothelium dependent control of vascular tone.

V. Inducing neurogenesis in the hippocampus through MC4R, as has been shown with afamelanotide in preclinical studies, improving memory and learning, and even reversing amnesia and memory reconsolidation impairment.

VI. Potentially enhancing the plasticity of the brain and overall recovery as MC stimulation might favour neuronal repair.

At therapeutic dose, afamelanotide has a limited ability to cross the BBB. However, following AIS, the BBB is disrupted, and MCs are expected to cross the BBB and also bind to the melanocortin 4 receptor (MC4R).

This broad understanding of the potential of MCs – as well as specific work done with afamelanotide – in stroke and other CNS models provided CLINUVEL with a strong justification to establish its clinical program.

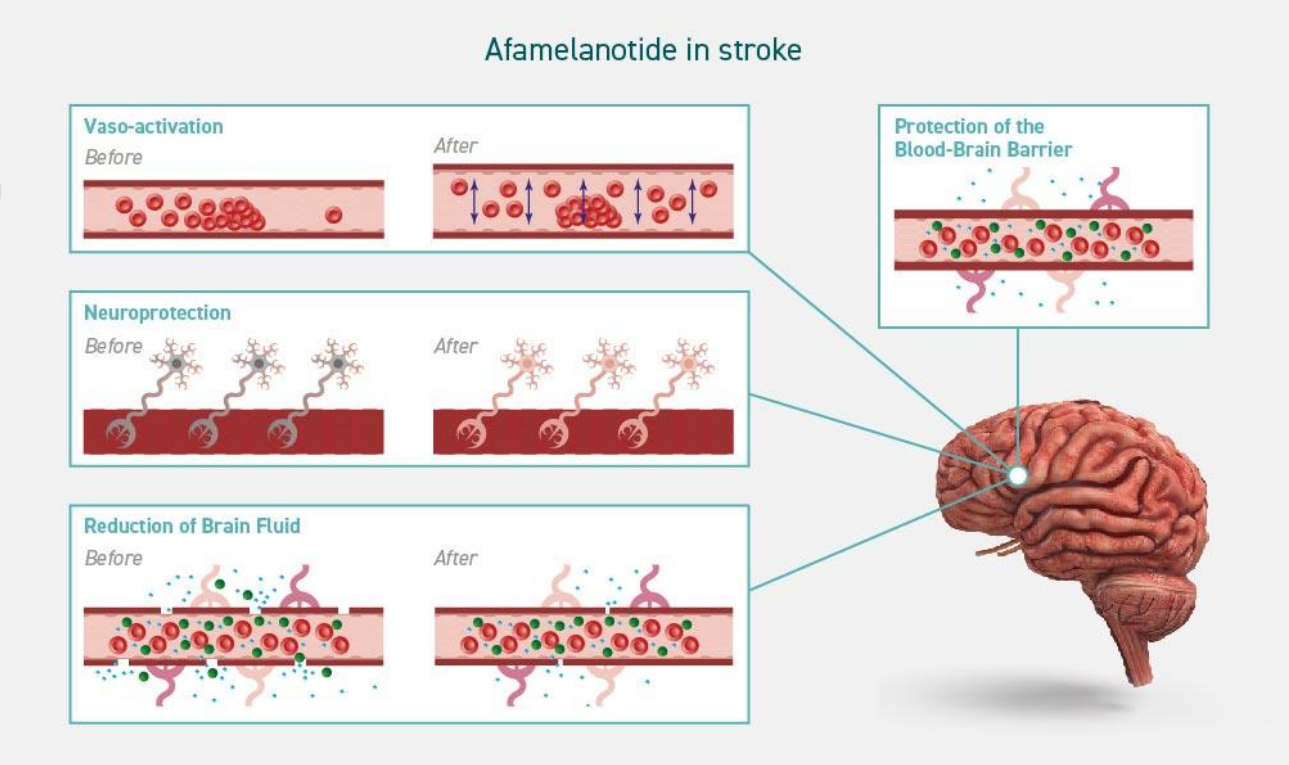


***Figure 3*** *Mechanisms of action of afamelanotide in stroke*

## Concluding statement

Investigation into the use of afamelanotide in stroke is founded upon a body of research supporting the drug's safety and potential efficacy in this largely untreated brain disorder. CLINUVEL's goal is to understand the optimal method and dose by which stroke patients could be treated in an emergency setting.

CUV803 will provide insight into the safety profile of PRÉNUMBRA® Instant in AIS patients and may lay the foundation for afamelanotide as a potential treatment for AIS. This aligns closely with CLINUVEL's core mission, innovating novel solutions for unmet patient and unaddressed healthcare needs. With AIS accounting for around 85% of the 15 million strokes suffered worldwide each year, any progress in developing new treatments for the indication has the potential to have a significant impact on patient outcomes and wellbeing. CLINUVEL is dedicated to developing new treatment methods and delivering high-quality healthcare.

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**About CLINUVEL PHARMACEUTICALS LIMITED**

CLINUVEL (ASX: CUV; ADR LEVEL 1: CLVLY; Börse Frankfurt: UR9) is a global specialty pharmaceutical group focused on developing and commercialising treatments for patients with genetic, metabolic, systemic, and life-threatening, acute disorders, as well as healthcare solutions for specialized populations. As pioneers in photomedicine and the family of melanocortin peptides, CLINUVEL's research and development has led to innovative treatments for patient populations with a clinical need for systemic photoprotection, assisted DNA repair, repigmentation and acute or life-threatening conditions who lack alternatives.

CLINUVEL's lead therapy, SCENESSE® (afamelanotide 16mg), is approved for commercial distribution in Europe, the USA, Israel, and Australia as the world's first systemic photoprotective drug for the prevention of phototoxicity (anaphylactoid reactions and burns) in adult patients with erythropoietic protoporphyria (EPP). Headquartered in Melbourne, Australia, CLINUVEL has operations in Europe, Singapore, and the USA. For more information, please go to https://www.clinuvel.com.



**Authorised for ASX release by the Board of Directors of CLINUVEL PHARMACEUTICALS LTD**

**Head of Investor Relations**
Mr Malcolm Bull, CLINUVEL PHARMACEUTICALS LTD

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**Forward-Looking Statements**

This release contains forward-looking statements, which reflect the current beliefs and expectations of CLINUVEL's management. Statements may involve a number of known and unknown risks that could cause our future results, performance, or achievements to differ significantly from those expressed or implied by such forward-looking statements. Important factors that could cause or contribute to such differences include risks relating to: our ability to develop and commercialise pharmaceutical products, the COVID-19 pandemic affecting the supply chain for a protracted period of time, including our ability to develop, manufacture, market and sell biopharmaceutical products; competition for our products, especially SCENESSE® (afamelanotide 16mg); our ability to achieve expected safety and efficacy results through our innovative R&D efforts; the effectiveness of our patents and other protections for innovative products, particularly in view of national and regional variations in patent laws; our potential exposure to product liability claims to the extent not covered by insurance; increased government scrutiny in either Australia, the U.S., Europe, China and Japan of our agreements with third parties and suppliers; our exposure to currency fluctuations and restrictions as well as credit risks; the effects of reforms in healthcare regulation and pharmaceutical pricing and reimbursement; that the Company may incur unexpected delays in the outsourced manufacturing of SCENESSE® which may lead to it being unable to supply its commercial markets and/or clinical trial programs; any failures to comply with any government payment system (i.e. Medicare) reporting and payment obligations; uncertainties surrounding the legislative and regulatory pathways for the registration and approval of biotechnology based products; decisions by regulatory authorities regarding approval of our products as well as their decisions regarding label claims; any failure to retain or attract key personnel and managerial talent; the impact of broader change within the pharmaceutical industry and related industries; potential changes to tax liabilities or legislation; environmental risks; and other factors that have been discussed in our 2022 Annual Report. Forward-looking statements speak only as of the date on which they are made, and the Company undertakes no obligation, outside of those required under applicable laws or relevant listing rules of the Australian Securities Exchange, to update or revise any forward-looking statement, whether as a result of new information, future events or otherwise. More information on the forecasts and estimates is available on request. Past performance is not an indicator of future performance.

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