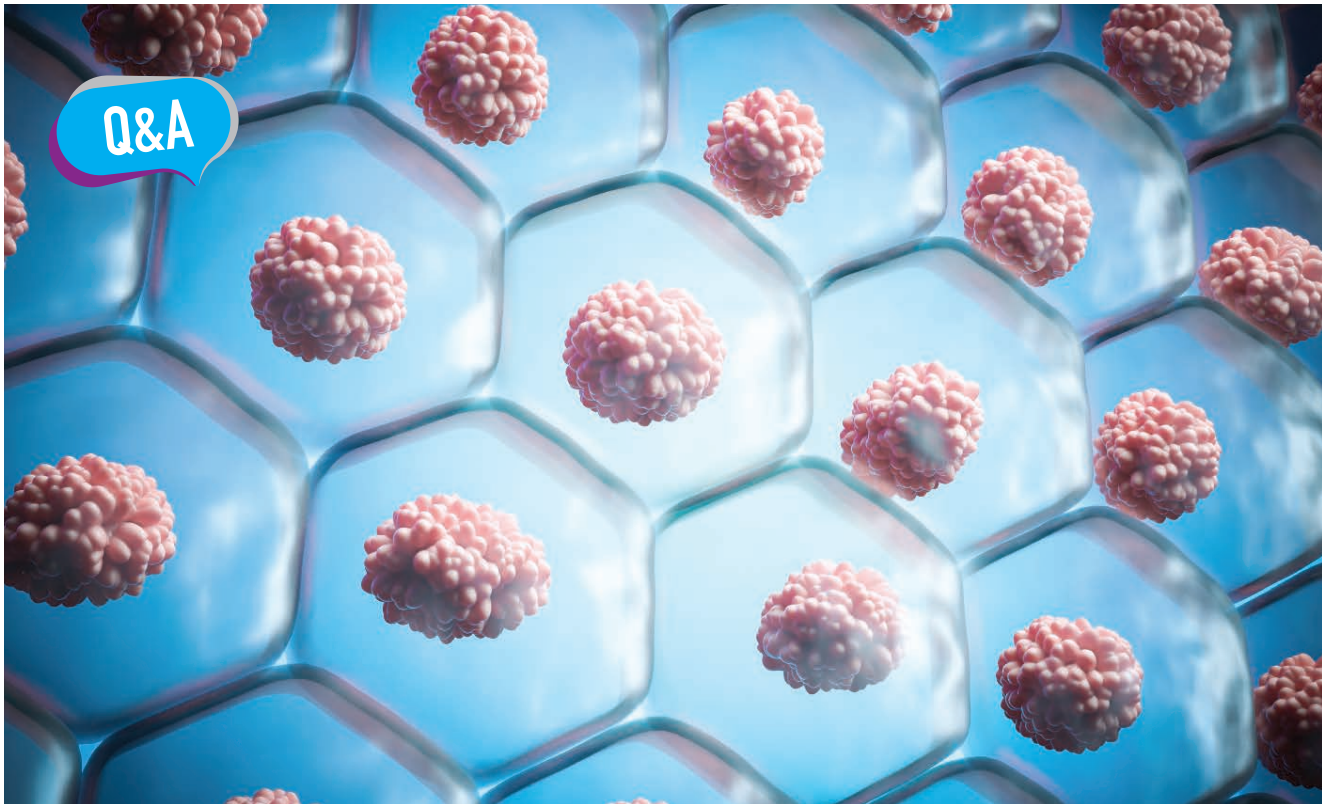


Promising initial clinical data for drug-resistant epilepsy

Neurona Therapeutics discuss their an investigational NRTX-1001, an investigational regenerative neural cell therapy for drug-resistant focal epilepsy derived from human pluripotent stem cells.

DTR HAD THE opportunity to interview Dr Cory R Nicholas, CEO of Neurona Therapeutics, about his company's exciting preclinical data on NRTX-1001, a regenerative neural cell therapy candidate derived from human pluripotent stem cells that is being investigated for drug-resistant focal epilepsy. The company presented promising preclinical and intial clinical findings at the Annual Meeting of the International Society for Stem Cell Research. In its preclinical studies, in the intrahippocampal kainate mouse model of chronic focal epilepsy, NRTX-1001 demonstrated a significant reduction in seizure frequency, with the fully-differentiated neural cells, called interneurons, secreting the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) to silence seizure activity in the epileptic region of the brain. These compelling results have paved the way for the clinical trial, which aims to evaluate the safety and efficacy of NRTX-1001 in patients with drug-resistant mesial temporal lobe epilepsy. If successful, the therapy has the potential to offer a non-destructive cell therapy option, with hopes of improving patients' quality of life and providing relief from drug-resistant focal seizures.

The press release mentions that NRTX-1001 is a regenerative neural cell therapy candidate derived from human pluripotent stem cells. Could you elaborate on the preclinical research that supported the development of NRTX-1001, including the selection and characterisation of the neural cell type and the rationale behind using human pluripotent stem cells?

The derivation of NRTX-1001 is based on years of research by Neurona cofounders Dr John Rubenstein, Dr Arturo Alvarez-Buylla, Dr Arnold »

> *The therapy has the potential to offer a non-destructive cell therapy option, with hopes of improving patients' quality of life and providing relief from drug-resistant focal seizures*

Kriegstein and current CEO, Dr Cory R Nicholas, PhD at UCSF, into the development and function of neural stem cells in the medial ganglionic eminence (MGE). The MGE, a brain structure that vanishes before birth, is the source of various specialised neuronal and glial cell lineages that populate the developing forebrain. The MGE is the predominant source of forebrain neurons that secrete the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). Local circuit GABAergic neurons, or interneurons, migrate extensively from the MGE, integrate into the overlying hippocampus and cortex, and balance excitatory neural activity. This inhibitory interneuron lineage is essential for regulating neural circuits and is known to be affected in multiple neurological disorders, including epilepsy. The founders wanted to develop a therapeutic cell strategy to replace the affected interneurons, rebalance neural activity and repair the nervous system.

When host neurons are evoked or hyperexcitable, as in epilepsy, they activate the grafted interneurons, which in turn then secrete GABA to inhibit the hyperactive host neurons

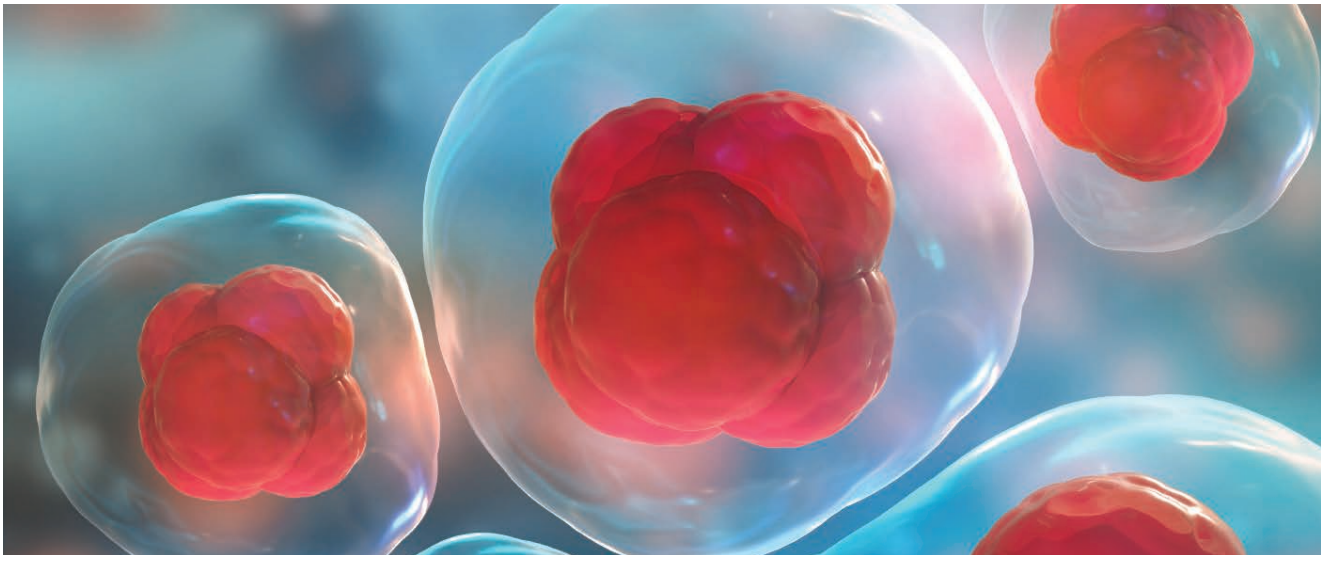
Early studies at UCSF demonstrated that cells taken from prenatal MGE tissue can replace the affected interneurons, and were efficacious in multiple preclinical models of epilepsy, Alzheimer's, Parkinson's and neuropathic pain.

For this to be a therapeutic however, a consistent, scalable, sustainable supply of human cells is required to support future clinical and commercial development. Neurona scientists selected an appropriate human pluripotent stem cell line and established proprietary methods to manufacture post-mitotic pallial MGE-type GABAergic interneurons, called NRTX-1001, from this line. Multiple GMP-compliant product lots of NRTX-1001 have been successfully produced in Neurona's in-house cGMP manufacturing facility. The development of the cell derivation method and the extensive molecular and functional characterisation of the interneurons are detailed in the company's first publication, which is in press and will be published in October in a high-impact peer-reviewed journal.

Can you provide insights into the preclinical studies conducted to evaluate the safety and efficacy of NRTX-1001 for mesial temporal lobe epilepsy (MTLE)? What animal models or *in vitro* models were utilised, and what key findings emerged from these preclinical investigations?

Neurona has generated promising data in a drug-resistant model of chronic mesial temporal lobe epilepsy (MTLE), demonstrating that single-dose NRTX-1001 administration into the temporal lobe stably suppresses focal seizures, significantly reduces temporal lobe pathology, improves memory and increases survival of the model. In dose finding studies, NRTX-1001 was found to be efficacious and safe in this model across a broad range of doses. Biodistribution studies with histological and qPCR-based analyses of central and peripheral tissues confirmed that NRTX-1001 cells were persistent and restricted to the hippocampus in the temporal lobe. The cells were persistent but did not multiply, and no off-target human cells were observed.

Daily observations and assessments of histopathology, haematology and clinical chemistries at multiple time points post-transplantation did not indicate adverse behavioural effects, or local or systemic dose-limiting toxicities related to NRTX-1001 treatment such as dizziness, sedation and ataxia which are the most common adverse effects of systemically administered GABA-potentiating antiseizure drugs. Furthermore, IND-enabling preclinical studies demonstrated accurate delivery of NRTX-1001 using the clinical delivery system.



The regenerative neural cells in NRTX-1001 are intended to integrate and innervate on-target, leading to the silencing of seizure activity in the epileptic region of the brain. Could you explain the specific mechanisms of action that were identified and validated during the preclinical research, demonstrating how these neural cells effectively suppress seizures?

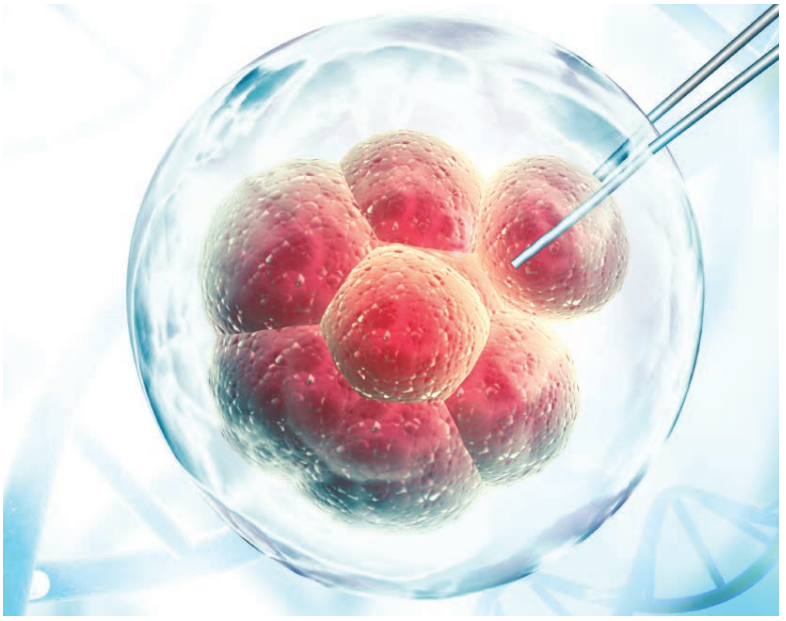
The grafted interneurons establish synaptic connections with host neurons. When host neurons are evoked or hyperexcitable, as in epilepsy, they activate the grafted interneurons, which in turn then secrete GABA to inhibit the hyperactive host neurons.

The press release mentions that NRTX-1001 is designed to provide long-term repair of dysfunctional neural networks for multiple neurological disorders. Based on the preclinical research, what evidence supports the potential applicability of NRTX-1001 beyond MTLE and its potential as a regenerative cell therapy candidate for other neurological disorders?

As NRTX-1001 is an investigational regenerative approach, we believe that it had potential applications in focal epilepsies beyond unilateral MTLE, such as bilateral MTLE and neocortical focal epilepsy that are not suitable for current standard of care destructive treatments that include resection and laser ablation. Beyond epilepsy, Neurona plans to advance clinical development of the NRTX-1001 interneurons for Alzheimer's disease. Many people with Alzheimer's disease have subclinical electrical discharges, like epileptic seizures, that begin in the temporal lobe (a memory centre) of the brain, have been hypothesised to potentiate disease progression, and are associated with more rapid cognitive decline, compared to people without these electrical discharges. Preclinical studies in models of Alzheimer's disease are ongoing to investigate whether NRTX-1001 can potentially normalise neural activity in the temporal lobe and improve memory.

Neuropsychological testing revealed potential memory improvements in patients who received NRTX-1001. Could you provide details on the preclinical studies or models that investigated the effects of NRTX-1001 on memory function, and the specific findings that supported the observation of memory improvement?

One of the comorbidities of drug-refractory epilepsy is impaired neurocognition, which can be exacerbated by resection or ablation surgeries that are temporal lobe destructive. In the ongoing open-label NRTX-1001 trial, it is encouraging that the first two patients showed preliminary signs of potential verbal and spatial memory improvement thus far. In the preclinical model of MTLE, cell-transplanted epileptic mice learned faster in a spatial memory test compared to vehicle-treated mice.



How did the preclinical data contribute to the decision to move forward with the clinical trial of NRTX-1001 for MTLE? Were there any specific efficacy, safety or mechanistic insights from the preclinical research that were particularly influential in advancing the development of NRTX-1001 to human trials?

MTLE is the most common type of focal epilepsy in adults. For those whose seizures are drug-resistant, treatment with anti-seizure medicines or neurostimulation rarely provides seizure-freedom. Some people may be candidates for surgery to remove or ablate the temporal lobe, however these procedures carry the risk of significant adverse effects. Furthermore, many patients are not eligible for the current surgeries based on the precise location, and number, of their seizure foci. We believe NRTX-1001 has the potential to address both those who are, and are not, candidates for current surgical treatments.

To date, the safety profile across multiple dosing range of NRTX-1001 has been favourable without dose limiting toxicity. The promising results we saw in pre-clinical studies, including profound effects on seizure reduction from a single dose as well as improvements in hippocampal pathology and memory, and reduced mortality were compelling reasons to advance NRTX-1001 into human clinical trials with drug-resistant MTLE.

Dr Cory Nicholas

Cory is a Founder and the Chief Executive Officer at Neurona Therapeutics.
Cory continues to serve as the Chief Scientific Officer at the company. Before Neurona, he was a faculty member in the Department of Neurology at the University of California, San Francisco where he investigated human cortical interneuron development.
Cory pioneered methods to derive interneuron precursors from human pluripotent stem cells, and he evaluated the therapeutic potential of interneuron cell therapy in multiple preclinical models of neurological disease. He maintains an adjunct faculty appointment at the University. Dr Nicholas's post-doctoral work was conducted at UCSF. His pre-doctoral research at both UCSF and Stanford University studied germline stem cells and gametogenesis. He received his bachelor's degree from the University of California, Berkeley.