A Diverse Portfolio of Stem Cells: Opening New Opportunities for Cell-based models and Discovery

Summary

- **Diverse Neural Portfolio**: Over thirty human neural stem cell lines developed, conditionally immortalised and available for licensing.
- **Controlled Cell Behaviour**: Engineered with a chemical switch for controlled proliferation and differentiation via c-myc technology.
- **Stability:** Genetically and phenotypically stable with normal, stable karyotypes; includes both male and female donor-derived lines.
- **Differentiation Capacity:** Capable of trilineage differentiation into various CNS cell types.
- **Research-Validated:** Supported by peer-reviewed publications and demonstrated performance.
- **Broad Applications:** Ideal for use in drug screening, disease modelling, and other preclinical research.
- **Additional Cell Lines:** Over twenty liver and pancreatic stem cell lines also available.

Conditional Immortalisation

A comprehensive catalogue of over 50 immortalised human stem cell lines, developed using the same immortalisation technology utilised for ReNeuron's clinical programmes. These research grade lines originate from various human tissues and are readily available for immediate use in R&D.

Available cell lines:

- CNS focused – Multiple Neural stem cell (NSC) lines derived from multiple regions of the CNS including cortex, striatum, hippocampus, ventral mesencephalon, cerebellum and spinal cord
- Non-CNS cell types – Liver, Pancreatic and Retinal.

These immortalised cells offer several key advantages over other cell lines, such as growth characteristics (retention of replicative capacity), consistent population doubling rates, genetic and phenotypic stability and well-established growth conditions.

The cell lines described here are engineered to express the growth-promoting c-myc gene, which is fused to a mutant oestrogen receptor, ERTAM. The fusion construct c-mycER^{TAM}, allows precise regulation of cell proliferation through the tamoxifen metabolite 4-hydroxytamoxifen (4-OHT). In the presence of 4-OHT, the cells maintain stable, long-term growth. Conversely, withdrawal of 4-OHT in vitro, leads to a cessation of proliferation and a shift towards a differentiated phenotype.

These features make the cell lines a robust and versatile platform for a variety of applications, including disease modelling, drug screening, and developmental biology studies.

Neural Stem Cell Lines:

ReNeuron has established immortalised human neural stem cell lines (hNSC) that can be maintained over multiple passages under defined culture conditions and retain multipotency. Critically, these cells can be induced to differentiate on demand, providing a versatile tool for neurobiology.

The hNSCs were isolated postmortem from human foetal tissue (10–14-weeks gestation) and are maintained on substrate-coated culture vessels (laminin or fibronectin) using a defined, serum free medium ("Human Media") supplemented with basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF). Alternatively, these lines can be cultured in commercially available, ready-to-use media supplemented with 4-OHT, which supports 4-OHT dependent proliferation. Under these conditions, all cell lines exhibit a consistent doubling time of 2-3 days.

NSC Characterization:

Phenotype

The phenotype of these lines has been profiled using ICC analysis in the presence and absence of growth factors and 4-OHT, to assess the expression of key neural stem cell markers as well as markers indicative of mature, differentiated cell types.

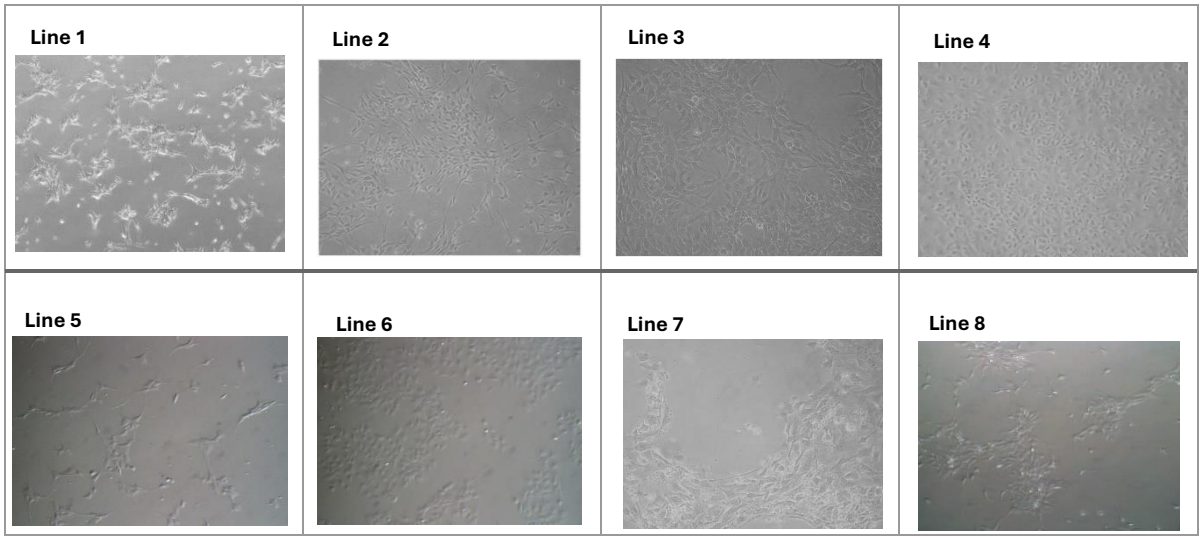

Table 1. *Images demonstrating the morphology of eight selected conditionally immortalised ReNeuron NSCs.*

Karyotype, Serology and Clonality

Karyotyping analysis was performed on all cell lines to determine sex and to screen for potential genetic abnormalities (Table 2). In addition, maternal serology was conducted to confirm that donors were free from major infections.

					Multipotency Potential- Neural Lineages Post- *In vitro* Differentiation		
Line	**Donor**	**Karyotype**	**Media**	**Culture Substrate**	**Neuron**	**Astrocyte**	**Oligodendrocyte**
1	1	Male, Normal	HM +GF +4-OHT	Laminin	Y	Y	Y
2	2	Male, Normal	HM +GF +4-OHT	Fibronectin	Y	Y	ND
3	3	Male, Normal	HM +GF +4-OHT	Laminin	Y	Y	Y
4	4	Female, Normal	HM +GF +4-OHT	Laminin	Y	Y	ND
5	5	Male, Normal	HM +GF +4-OHT	Laminin	Y	Y	Y
6	6	Female, Normal	HM +GF +4-OHT	Laminin	Y	Y	Y
7	7	Female, Normal	HM +GF +4-OHT	Laminin	Y	Y	ND
8	8	Female, Normal	HM +GF +4-OHT	Laminin	Y	Y	Y

Table 2. *Table to demonstrate a selection of the distinct foetal-derived neural stem cells (NSCs) available. NSCs are isolated from distinct donors and demonstrate their multipotency for distinct neural lineages. ND = Not determined.*

Potential to Target New Discovery Opportunities:

The distinct hNSC lines available represent a valuable and diversified portfolio with broad applicability across biomedical research and therapeutic development. These lines offer a robust and reproducible platform for generating accurate, disease-relevant models.

Potential Applications include:

- Phenotypic and genetic screening
- Target engagement and validation
- Drug and biomarker discovery
- Cytotoxicity evaluation
- Lead optimisation and efficacy evaluation
- Development of 2D and 3D neural cell models
- Genetic engineering for disease modelling, including the introduction of disease-specific mutations and the availability of genetically matched controls.
- Generation of induced pluripotent stem cells (iPSCs) (ReNeuron IP)

These NSC lines enable the development of reliable cell-based assays for screening and efficacy studies across multiple neurological indications, including Alzheimer's disease, Parkinson's disease, stroke, traumatic brain injury (TBI), and spinal cord injury, thereby supporting the advancement of next-generation therapeutics.

Other ReNeuron Stem Cell lines also available:

In addition to neural stem cells (NSCs), ReNeuron have developed over 20 distinct conditionally immortalised cell lines derived from human foetal liver and pancreatic tissues. These lines, like the NSCs, have been engineered using the recombinant c-mycER^{TAM} construct, enabling controlled proliferation in the presence of 4-hydroxytamoxifen (4-OHT).

Initial characterisation and phenotypic profiling have confirmed the expression of key hepatic and pancreatic markers, underscoring their potential as valuable non-neural stem cell models. These lines represent a promising platform for further development and application in research areas such as diabetes, metabolic disorders, and organ-specific disease modelling.

ReNeuron Publications:

In addition to ReNeuron's internal characterisation data, many of these neural stem cell (NSC) lines have a well-established track record in the scientific literature. Extensive publications highlight the unique capabilities of the conditionally immortalised cell technology, including comprehensive characterisation and a wide range of applications. To date, over 50 peer-reviewed papers have been published using the conditionally immortalised stem cells developed by ReNeuron, demonstrating their utility in both basic and translational research.

Some examples of these include:

1) Amemori, T., Romanyuk, N., Jendelova, P. et al. Human conditionally immortalized neural stem cells improve locomotor function after spinal cord injury in the rat. Stem Cell Res Ther 4, 68 (2013). https://doi.org/10.1186/scrt219
2) Anacker C, Zunszain PA, Cattaneo A, Carvalho LA, Garabedian MJ, Thuret S, Price J, Pariante CM. Antidepressants increase human hippocampal neurogenesis by activating the glucocorticoid receptor. Mol Psychiatry. 2011 Jul;16(7):738-50. doi: 10.1038/mp.2011.26. Epub 2011 Apr 12. PMID: 21483429; PMCID: PMC3121947.
3) Anderson, G.W., Deans, P.J.M., Taylor, R.D.T. et al. Characterisation of neurons derived from a cortical human neural stem cell line CTX0E16. Stem Cell Res Ther 6, 149 (2015). https://doi.org/10.1186/s13287-015-0136-8
4) Borsini A, Di Benedetto MG, Giacobbe J, Pariante CM. Pro- and anti-inflammatory properties of interleukin (IL6) in vitro: relevance for major depression and for human hippocampal neurogenesis. Int J Neuropsychopharmacol. 2020 Jul 29;23(11):738–50. doi: 10.1093/ijnp/pyaa055. Epub ahead of print. PMID: 32726406; PMCID: PMC7745251.
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11) Cocks, G., Romanyuk, N., Amemori, T. et al. Conditionally immortalized stem cell lines from human spinal cord retain regional identity and generate functional V2a interneurons and motorneurons. Stem Cell Res Ther 4, 69 (2013). https://doi.org/10.1186/scrt220
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