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Title

Human pluripotent derived Auditory Neuron Progenitors (LCTANP1) for the treatment of auditory neuropathy spectrum disorder (ANSD)

Background:

Loss of auditory nerve cells can lead to auditory neuropathy, even when the hair cells and the cochlear nucleus remain intact. Cell-based therapy for replacing lost or dysfunctional auditory neurons may restore hearing in these cases. In severe cases, where both hair cells and many neurons are lost, the degree of success of a cochlear implant procedure may be enhanced by repopulating the cochlea with transplanted, functional auditory neurons. We developed a novel proprietary differentiation process to manufacture LCTANP1 composed of Auditory Neuron Progenitors (also termed otic neuronal progenitors) from pluripotent human stem cells. As an integral process the LCTANP1 manufacturing process and cells were characterized by biological and functionally relevant sets of markers, using different quantitative methods that we newly developed and customized including functional in vitro assays. LCTANP1 cells were transplanted via direct administration into the cochlea of guinea pigs in which neurons were previously eliminated via exposure to ouabain. The survivability of engrafted LCTANP1 when transplanted into the deafened cochlea of guinea pigs was assessed.

Methods:

LCTANP1 production - The differentiation process includes the expansion of a clinical grade line of human pluripotent stem cells, a series of differentiation cues that are delivered under specific culture conditions, in specific time frames, harvesting the final Auditory Neuron Progenitors cells and lastly, cryopreservation in a ready to administer format.

Development of analytical biomarkers included the analysis of specific protein marker expression by flow cytometry, and immunofluorescence and expression profiles, including RNA sequencing.

Functional assays were developed to measure neuronal properties of LCTANP1. Functional assays test cells' ability to elicit calcium influx (which plays an important role in multiple signaling cascades within auditory neurons), in response to glutamatergic agonists in a time-dependent mannerThe ability to express synaptic markers which plays a role in synapse formation of auditory neurons with hair cells .

The cochleae of eight ouabain-treated guinea pigs were infused with 5 µl of red fluorescent labeled LCTANP1 cells via a cochleostomy into the scala tympani, or via a needle inserted into the modiolus, both procedures performed at the base of the cochlea. Seven days later, animals were euthanized and labeled LCTANP1 cells were visualized within the cochlea using a fluorescence stereoscope. Cochlea were processed for immunohistochemistry, dissected, and imaged for assessment of LCTANP1 cells.

Results:

LCTANP1 cells were successfully manufactured at scale, met pre-set release criteria, and demonstrated relevant activity in *in-vitro* functional tests. LCTANP1 cells were cryopreserved in a ready-to-administer, thaw and inject format and were successfully thawed, successfully transplanted and survived in an *in-vivo* Guinea pig model for at least 7 days. LCTANP1 cells are currently being evaluated in a functional model of hearing simultaneous with additional manufacturing enhancements.

Conclusions:

LCTANP1 is a novel cell-based product composed of Auditory Neuron Progenitors derived from clinical grade pluripotent stem cells. LCTANP1 completed initial CMC and Preclinical POC and is ready for the next stages of preclinical development.