OTOSTEM

HUMAN STEM CELL APPLICATIONS FOR
THE TREATMENT OF HEARING LOSS

PUBLISHABLE SUMMARY

3rd PERIODIC REPORT

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Summary description of project context and objectives

The lack of human otic cell models represents a significant roadblock hampering the development of drug-based or cell-based therapies. Hearing impairment is the most frequent human sensory deficit and is mainly caused by the irreversible loss of neurosensory cells in the cochlea. OTOSTEM addresses this urgent and unmet medical need for causal hearing loss therapies by focusing on human stem cell technology. We will generate human inner ear models as a platform for the development of novel therapies for sensorineural hearing loss. Purified otic progenitor cells from various human stem cell sources will be the core of this technology. This will provide the basis for preclinical and clinical development of drug and cell-based therapies.

Concept

Our ability to hear depends entirely on our auditory receptors – the sensory hair cells and their associated neurons that reside in the cochlear part of the inner ear (Figure 1). Mechanical signals (acoustic waves) are transformed in the organ of Corti into electric signals (action potentials). Hair cells activated by the movement of the cochlear fluid release chemical messengers, which stimulate the auditory nerve carrying the information to the brain for processing. The exquisite sensitivity of the inner ear comes with the risk for damage for example by noise trauma, ototoxic drugs, infections, age related degeneration and genetic causes. Once lost, the neurosensory cells of the ear are not replaced. This, in turn results in chronic hearing impairment, a devastating and highly prevalent disorder of infancy and adulthood with widespread implications for the individual and society as a whole. Adult hearing loss alone ranks among the five leading causes of burden of disease in Europe, entailing enormous socio-economic costs. Prosthetic treatment with hearing aids

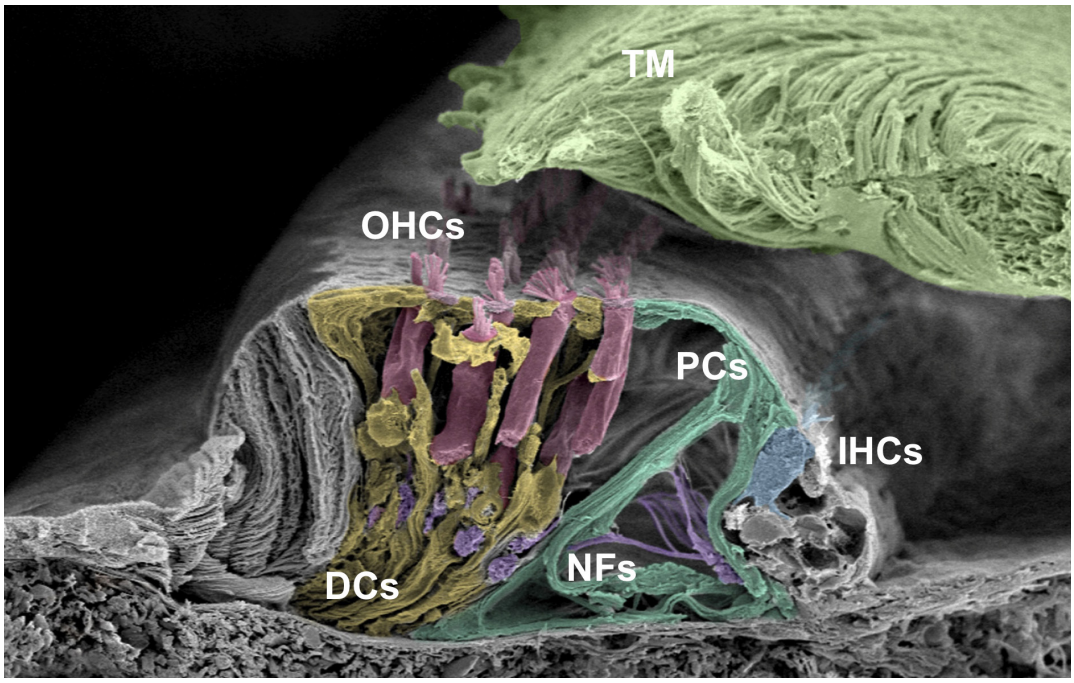

Figure 1: Pseudo-coloured scanning electron micrograph of the organ of Corti in a human cochlea. Outer hair cells (OHCs-red); inner hair cells (IHCs - blue); tectorial membrane (TM – green); Deiters cells (DCs – yellow); Pillar cells (PCs – turquoise); nerve fibres (NFs – purple). The image was kindly provided by Professor Helge Rask-Andersen (University of Uppsala).

and cochlear implants is limited and reaches only every fifth patient. Due to the cause of the hearing loss – neurosensory cell loss – hearing aid amplification often fail to improve language comprehension and hence perform unsatisfactory.

Towards cell-based therapies (Figure 2), OTOSTEM evaluates defined cell populations intended for therapeutic use for tumorigenic potential and validate their functional properties and biological potency in appropriate *in vitro* models.

Towards drug-based therapies (Figure 2), cellular otic models are developed to the level of artificial sensory epithelia or "mini-ear" *in vitro* models mimicking the *in vivo* organ equivalent.

Otic cells have to be generated in sufficient numbers to allow screening in a high throughput/high content multi well plate format. In a subsequent step, these established and characterised cellular otic models are advanced into models of hearing loss. Exposure to ototoxic drugs allows for selective ablation of sensory hair cells and the establishment of "hearing loss in a dish" models for ototoxic drug screening.

One goal is to provide working assays for high throughput/content screens to the point that these assays can be used in the drug discovery setting represented by SMEs. Two therapeutic classes of drugs, one with otoprotective and the other one with otoregenerative effects are in the primary focus. Otoprotective effects aim to prevent cell death of human hair cells while otoregenerative effects aim at the replacement of lost human auditory neurons and hair cells.

Achievements

Controlling differentiation and proliferation in human otic stem cells

Human otic cell models are urgently needed for the development of cell- and drug-based hearing loss therapies. This principal technological hurdle was taken with a delay in the last funding period by the OTOSTEM consortium. Several approaches to generate human otic cells from different stem cell source (embryonic and inducible pluripotent cells, fetal and adult native human stem cells) were tested. A phenotypic

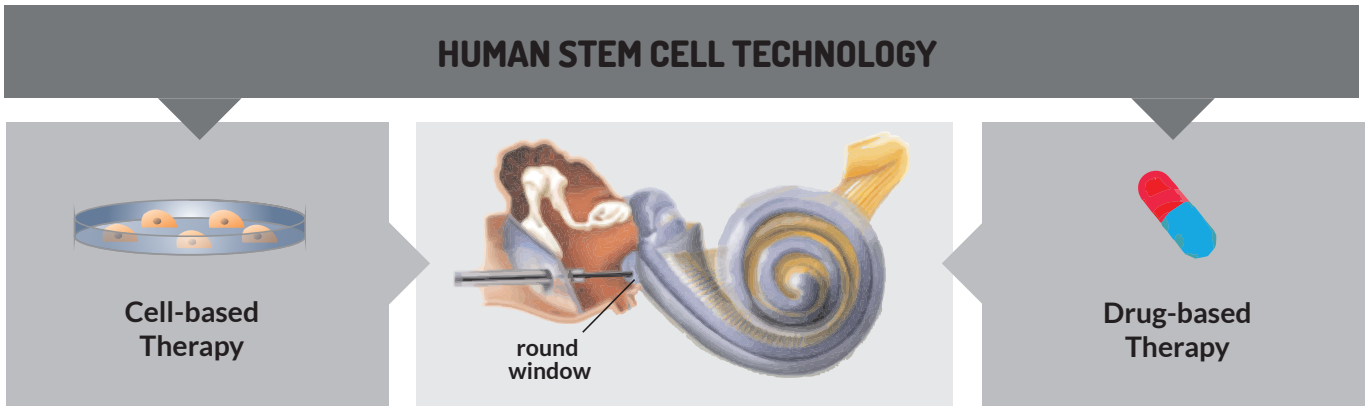

Figure 2: The two major applications for the therapeutic use of human stem cell technology for hearing loss are (i) direct cell based treatment by transplantation of human otic stem/progenitor cells into the cochlea and (ii) drug based treatment emanating from drug screening efforts for otoprotective and otoregenerative compounds. Both cells and drugs will be applied directly into the target organ – the cochlea –, which is self-contained and surgically accessible. An access route into the cochlear fluid space is provided through the round window membrane.

comparison, including a meta-analysis of gene expression data, was conducted to identify the source with the highest otic differentiation potential. Native human stem cells turned out to be the best source to generate sensory hair cell-like cells with rudimentary hair bundle-like structures. This novel human otic model may provide the basis for preclinical development of cell- and drug based therapies.

Furthermore, new guidance protocols to differentiate sensory or neuronal cell types from pluripotent stem cells were explored. Significant technical improvement to characterize and prospectively purify otic progenitor cells (OPCs) from human pluripotent stem cells was made. Positive and negative surface markers that may facilitate the purification of OPCs from heterologous cell populations by flow cytometry were identified and partially validated. These markers may improve and standardize the quality and purity of OPC populations to be used for cell-based therapies.

Significant improvements were reached in the advancement of a murine inner ear model. Cochlear otic progenitor cells were cultured and expanded more than 2000-fold using a cocktail of small molecules. The progenitor cells, in turn, gave rise to inner ear organoids with functional hair cells, proven by actin rich bundles, the molecular machinery for transduction, synapse formation, and specialized hair cell activity. This novel protocol leads to high yield in hair cells, dramatically reducing the number of experimental animals. The developed cocktail of compounds, once optimized for human cells, holds the promise to also improve the yield of hair cells in the human models.

Otic progenitor cells suitable for cell transplantation

For safe transplantation, stem cell-derived otic cells have to be thoroughly characterized regardless of their means of isolation and purity. It is essential to exclude tumorigenicity of the transplanted cells and to prove cell integration and survival. A long-term study was undertaken using highly purified human otic neuronal progenitors and delivered in the cochlea of deafened and immunodeficient gerbils. Hearing restoration was achieved and preserved for more than 30 weeks after transplantation, indicating that the transplanted cells integrated, survived and are safe. So far, no evidence of tumour formation was found in these animals as judged by whole-body magnetic resonance imaging. Although not yet completed, these results are very promising for future cell-based therapies.

A comprehensive characterization of the complement of ion channels expressed by cochlear neurons and lateral wall cells of human cochleae was presented using super-resolution structured illumination microscopy (SR-SIM).

Hearing loss in a dish model, ototoxicity, and drug screening

There is no quantitative assay for ototoxicity in humans. FDA-approved drugs bear the risk to cause hearing loss. Using human induced pluripotent stem cells (iPSCs) differentiated into the otic lineage, a quantitative human ototoxicity assay was developed and utilized. This assay was used to predict the toxicity of more than 2000 compounds (770 drugs approved by the FDA and 1280 pharmacological active compounds). We detected six compounds with ototoxic properties of which two could be validated

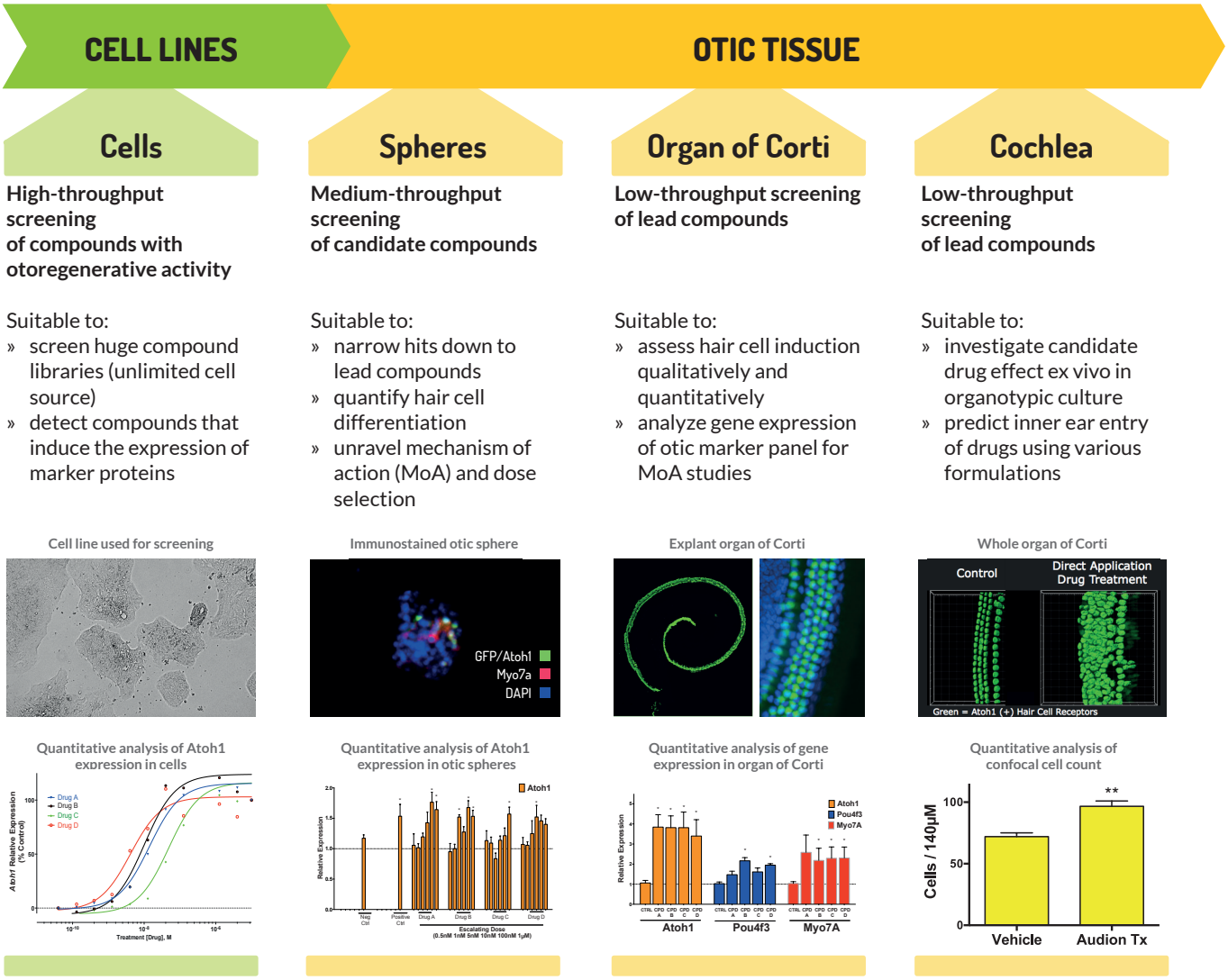

Figure 3: Screening cascade for drug discovery of OTOSTEM partner Audion Therapeutics BV. Overview of the in vitro *otic differentiation models. Shown here is the expanded platform of models used for comparative benchmarking capabilities for compound validation studies, screening and studying phenotypic conversion. The cascade starts with relative simple cell culture assays in which compounds are screened at high-throughput. Lead compounds are screened with more complex model systems closer to the* in vivo *situation.*

in murine and human otic models. Further investigations *in vitro* and *in vivo* are required before drug safety recommendations can be published.

Drug screening aiming at otoprotection and otoregeneration

During the current reporting period, bioassays to screen for compounds with otoprotective or otoregenerative activity were continuously improved. Due to the delay in the derivation of human otic cells from stem cells, some of these assays were developed using mouse cells, whilst others assays were based on human cells. By now, members of the consortium have established and validated tools for otic drug discovery ranging from relatively simple single cell to complex whole organ culture assays (Figure 3). These customizable *in vitro* assays offer multiple quantifiable parameters including gene expression and phenotypic characteristics of mature hair cells and supporting cells. They have facilitated the selection of potential drugs which have been further optimized to increase potency and specificity. The best performing compounds were validated at lower throughput

in more elaborate assay systems, closer to the *in vivo* situation. The murine organ culture assay, for example, was used to quantify the activity and to determine dose-response relationships of lead compounds. Further characterization of drug candidates included a broad biochemical safety panel (CEREP Screen) to evaluate development and therapeutic potential. Two of three identified compounds successfully passed these *in vitro* tests and were advanced to *in vivo* experiments.

In vivo investigations of stem cell and drug based therapies in hearing loss models

Substantial progress in the characterization and standardization of several *in vivo* rodent models for sensory hair cell or auditory neuronal loss was made. These hearing loss models cover a wide range of human hearing conditions. In the last period of the project, different surgical approaches were compared and refined to optimize the delivery of cells. The hearing status of the treated animals was quantified electrophysiologically and improvements in functional outcomes were assessed on the cellular level (Figure 4).

Compounds with otoregenerative and otoprotective activity were locally applied in *in vivo* experiments using hearing loss models. Protocols and formulations were developed for the safe and effective delivery of small molecule compounds into the inner ear. Pharmacokinetic measurement revealed that both lead compounds were absorbed by the inner ear and reached effective drug concentrations. Otoprotective and otoregnerative drug candidates were shown to partially restore auditory function of *in vivo* hearing loss models. One otoprotective drug candidate is currently in preclinical testing and shows promising results. A second drug targeting otoregenerative candidate drug has successful reached clinical testing.

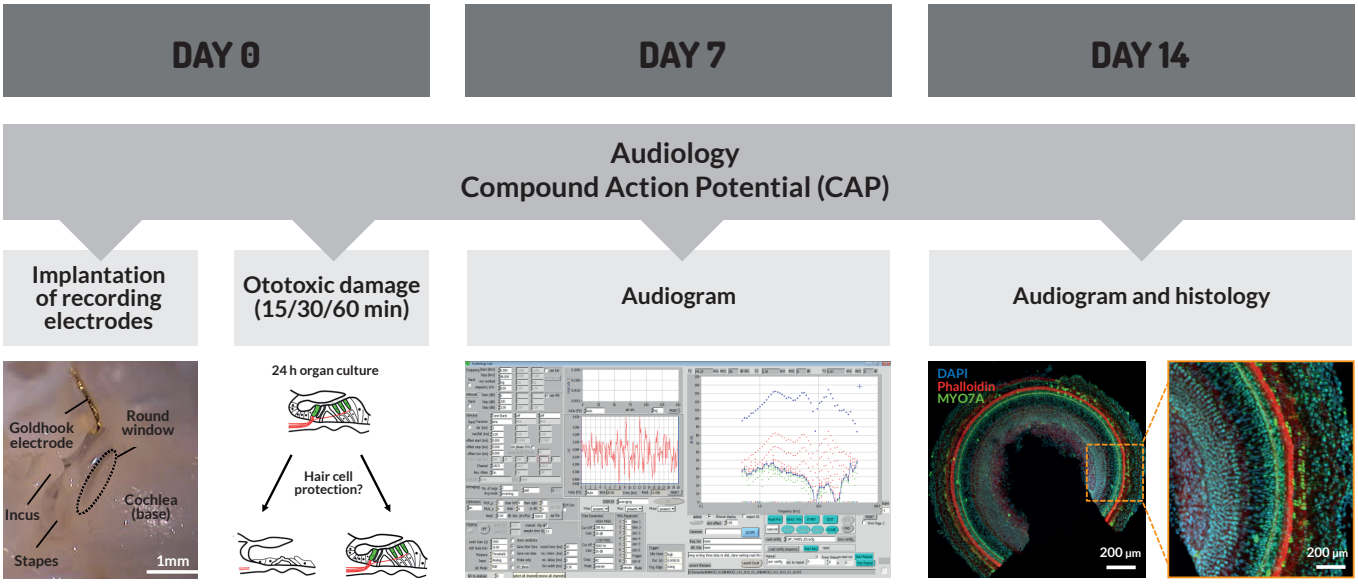

Figure 4: Schematic workflow of in vivo *drug testing experiment to investigate otoprotective compounds of OTOSTEM partner Acousia Therapeutics GmbH. The hearing status of the animals was assessed electrophysiologically by CAP measurements before and immediately after the ototoxic insult (day 0). Otoprotective test compounds were administered and measurements were taken at day 7 and day 14 for analysis. Improvements in functional outcomes were assessed on the cellular level by immunohistological techniques.*

Conclusion

During the third and last period of the OTOSTEM project, the majority of the proposed tasks were successfully completed. The partners collectively presented 16 talks and 11 posters at national and international scientific events in this reporting period. Ten peer-reviewed papers have been published within the last 12 months and further publications have been submitted or are in preparation. The scientific and personal interactions, which were made possible by the OTOSTEM project, generated new ideas and projects to fight hearing loss.

Peer-reviewed publications in scientific journals

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