

OTOSTEM



HUMAN STEM CELL APPLICATIONS FOR
THE TREATMENT OF HEARING LOSS

FINAL PUBLISHABLE SUMMARY

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The OTOSTEM project followed two major directions to implement a therapeutic use of human stem cell technology for hearing loss. First, OTOSTEM used human cells for cell-based treatment with purified cells transplanted into the cochlea. Second, OTOSTEM employed human stem cells to generate human otic cellular models to allow screening for ototoxic, otoprotective and otoregenerative compounds for drug-based treatments applied locally in the cochlea.

Substantial progress in the development of drug and cell-based therapies against hearing loss was reached by the consortium in the four years of the OTOSTEM project. Several approaches to generate human otic sensory and neuronal cell types from different stem cell sources were developed and compared. Surface markers to purify otic progenitor cells from heterologous cell populations were identified and partially verified. The functional properties and biological potency of cells intended for use in cell-based therapies was carefully assessed.

Otic drug screening assays were designed and implemented to enable drug safety studies and drug development capabilities. More than 2000 pharmacological active substances were tested for ototoxic properties and several candidates were identified and validated.

Two out of three newly identified potential drug candidates to treat hearing loss passed various *in vitro* and *ex vivo* preclinical tests. For further validation purposes, several *in vivo* rodent models for sensory hair cell or auditory neuronal loss were systematically characterized and standardized. Ultimately, the developed cell-based and drug based therapies were validated in *in vivo*.

Overall, OTOSTEM was – based on our own judgement – a successful project that significantly advanced the development of novel therapies against hearing loss. With the support of the Seventh Framework of the EU scientist from academia and industry translated basic research into clinical applications and tools.

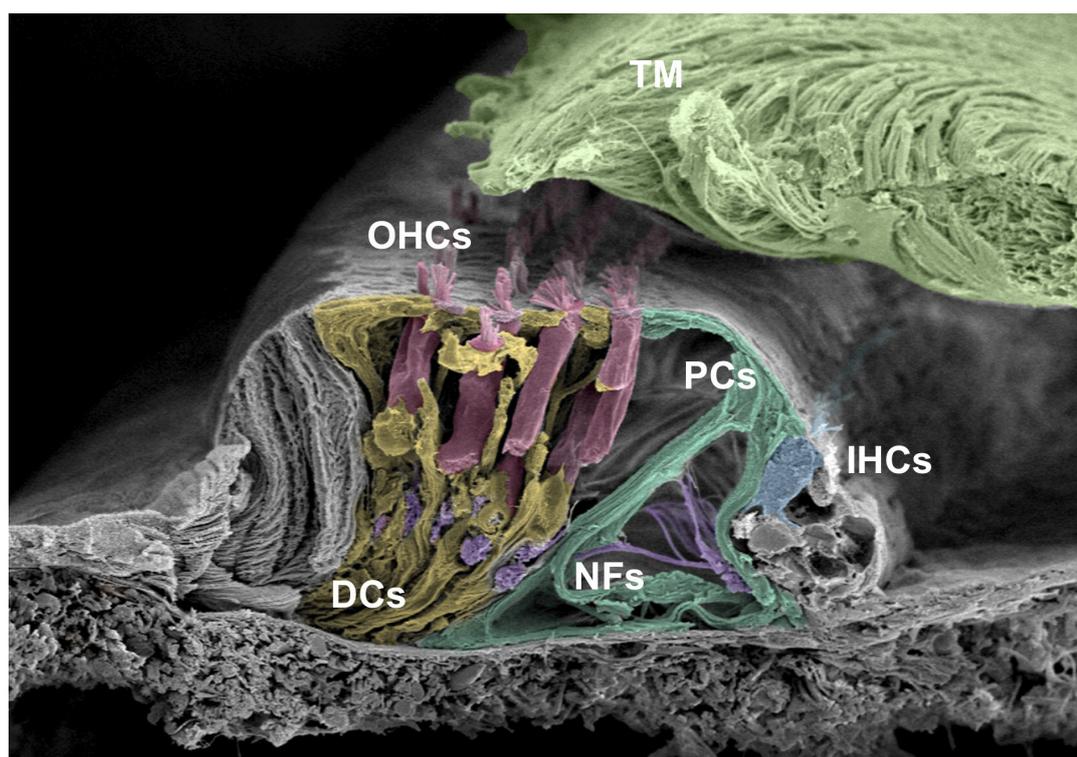


Figure 1: Pseudo-coloured scanning electron micrograph of the organ of Corti in a human cochlea. Outer hair cells (OHCs - red); inner hair cells (IHCs - blue); tectorial membrane (TM - green); Deiters cells (DCs - yellow); Pillar cells (PCs - turquoise); nerve fibres (NFs - purple). The image was kindly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

Summary description of project context and objectives

Hearing impairment represents the most frequent human sensory deficit. It is mainly caused by the irreversible loss of neurosensory cells in the cochlea. 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 residing in the organ of Corti are activated by the movement of the cochlear fluid and 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 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.

Reliable human otic cell models are urgently required for the development of drug-based or cell-based therapies against hearing loss.

The main goal of the OTOSTEM project was to address this unmet medical need for causal hearing loss therapies in a collaborative effort.

The focus was set on human stem cell technology to generate human inner ear models. It was envisioned to create a human platform for the development of novel therapies for sensorineural hearing loss. OTOSTEM perused two major applications for the therapeutic use of human stem cell technology for hearing loss: i) direct cell-based treatment emanating from human otic stem/progenitor cells into the cochlea and ii) drug-based treatment emanating from drug screening efforts for otoprotective and otoregenerative compounds (Figure 2). Both cells and drugs were 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.

Purified human otic progenitor cells (OPC) derived from stem cells were considered to be the core technology of the OTOSTEM project. The initial task was to identify a reliable source of OPCs that could be i) purified using surface marker ii) expanded *in vitro* and iii) differentiated to give rise to sensory hair cells, supporting cells or otic neuronal cell types. For this task, different sources of human stem cells were available: native stem cells from fetal and adult human otic tissues as well as embryonic or induced pluripotent stem cells (ESC or iPSCs). The stem cell source with the highest potential to differentiate into the otic lineages was thought to provide the basis for the subsequent tasks.

HUMAN STEM CELL TECHNOLOGY

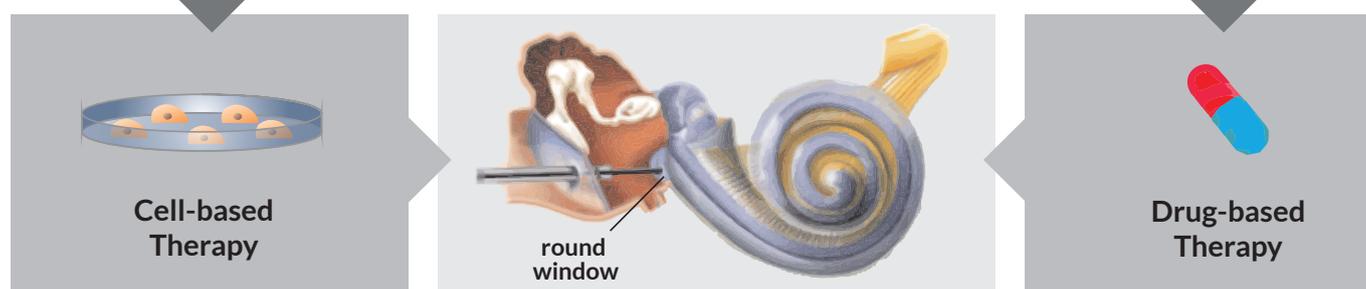


Figure 2: The two major applications for the therapeutic use of human stem cell technology for hearing loss are (i) direct cell based treatment by transplantation of human otic stem/progenitor cells into the cochlea and (ii) drug based treatment emanating from drug screening efforts for otoprotective 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.

OTOSTEM planned to evaluate OPCs intended for therapeutic use with respect to biological potency and tumorigenic potential in appropriate assays. Non-tumorigenic, enriched OPCs with the capacity to give rise to functional inner ear cell types serve as a basis for cell-based therapies. Another concern of the OTOSTEM project was to establish a novel quantitative ototoxicity assay based on human stem cells for drug screening and drug safety studies. This “hearing loss in a dish” model was intended as the working assays for high throughput screens used in the drug discovery setting represented by SMEs. Two therapeutic classes of drugs, one with otoprotective and the other one with otoregenerative effects were in the primary focus. Otoprotective effects aim to prevent cell death of human hair cells caused by drugs or noise, while otoregenerative effects aim at the replacement of lost human auditory neurons and hair cells. In a subsequent step, the candidate drugs and cell populations were advanced into *in vivo* models of hearing loss. *In vivo* cell transplantation of otic sensory or neuronal cell types as well as drug treatment studies were envisioned using animal models of auditory neuropathy, meningitis and drug induced hearing loss.

Description of main S & T results/foregrounds

Controlling differentiation and proliferation in human otic stem cells

The development of cell- and drug-based hearing loss therapies is hampered by the lack of human otic cell models. The OTOSTEM consortium overcame this principal technological hurdle in the last funding period. Several approaches to generate human otic cells from either pluripotent stem cells or native human stem cells were tested.

Embryonic or induced pluripotent stem cells (ESCs or iPSCs) were first differentiated into OPCs and in a subsequent step into otic cell types. Fetal and adult native stem cells from the inner ear instead were already predetermined to give rise to otic cell types. They had to be purified from a heterogeneous cell population and were then differentiated into mature otic cell types. Several guidance protocols to differentiate sensory or neuronal cell types from human stem cells were devised and explored. These protocols make use of morphogens or small molecule compounds to induce otic fate either by

the stepwise recapitulation of normal development or by direct conversion of cell fate. Positive and negative cell surface markers of OPCs were identified. They may facilitate the purification of defined cell populations by flow cytometry. Several other methods that enrich for otic cells from heterogeneous cell populations were explored. They proved to be less reliable and were not further investigated.

Finally, a phenotypic comparison, including a meta-analysis of gene expression data, among different partners in the consortium was conducted to identify the stem cell 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.

Nevertheless, the differentiation of pluripotent stem cells (ESCs and iPSCs) into OPCs remains to be of particular importance, because they represent an expandable source of stem cells. Large quantities of cells are needed for drug design especially when high throughput screens are planned. There is strong consensus in the consortium, that otic differentiation from pluripotent cell sources (ESCs and iPSCs) has the potential to give rise to otic progenitor cell (OPC) populations. It was demonstrated that the differentiation protocols used in this project lead to OPC-like cells that express several typical native markers. These cells are generated via a logic trajectory where pluripotency genes are becoming downregulated, followed by the transient expression of precursor cell markers, and ultimately otic markers. However,

the analyses also revealed that it is difficult to maintain pluripotent stem cell-derived OPC populations because they are inherently unstable. Whether this instability is based on the lack of commitment to the otic lineage or on adoption of a non-sensory otic fate remains to be investigated.

Significant improvements were accomplished in the 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 new protocol leads to high yield in hair cells, which dramatically reduces 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

Another aim of OTOSTEM was to evaluate the functional properties and biological potency of stem cells-derived otic cells intended for therapeutic use (Figure 2). For safe transplantation, candidate cell populations have to be thoroughly characterized regardless of their means of isolation and purity. It is essential to prove the functional integration and survival of transplanted cells and to exclude any tumorigenic activity in appropriate models.

In the first instance, the functionality of human iPSC-derived otic neuronal and sensory hair cells was analysed in *in vitro* experiments. The

electrophysiological recordings of a subpopulation of the human hair cell-like cells resembled those of mouse cochlear hair cells. Furthermore, the newly generated hair cells incorporated the dye FM1-43, an indicator for functional mechanotransduction channels. These data showed that otic neurons generated *in vitro* are rather mature and functional. The generation of mature hair cells with well-established stereocilia bundles remains to be improved.

To advance these functional experiments and get closer to the *in vivo* situation, a whole-organ culture system of murine cochlea was set up. In such model systems, cell survival and functional integration of candidate otic cell populations can be investigated, allowing the assessment of transplantation experiments *ex vivo*. The *ex vivo* survival time of the organ culture is currently not long enough; new strategies to overcome these limitations were developed and preliminary data were collected.

The safety of cell transplantation derived from stem cells was addressed in several *in vivo* experiments. The conducted transplantation experiments showed that a two-step purification (manual picking and cell sorting) prior to implantation of human ESCs-derived OPCs into immunosuppressed mice led to superior results than a single step protocol. The identified positive and negative surface markers may further improve

the purification processes of OPC. A long-term study was undertaken using highly purified human otic neuronal progenitors delivered to 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 encouraging for the development of cell-based therapies.

Our knowledge of the channel composition and electrophysiological properties of the mature human neuronal and sensory cell types is still limited. Protocols to maintain adult human Scarpa and spiral ganglion explants in culture enabling their *in vitro* characterisation were developed. Spontaneous or evoked action potentials were recorded from fetal or adult inner ear neurons using multi electrode arrays. Patch clamp recordings of Scarpa's ganglion neurons were set up, while the recording of vestibular hair cells remains to be established. Native Scarpa's ganglion cells were cryopreserved and banked for further studies. A comprehensive characterization of the ion channel composition expressed by cochlear neurons and lateral wall cells of human cochleae was presented using super-resolution structured illumination microscopy (SR-SIM).

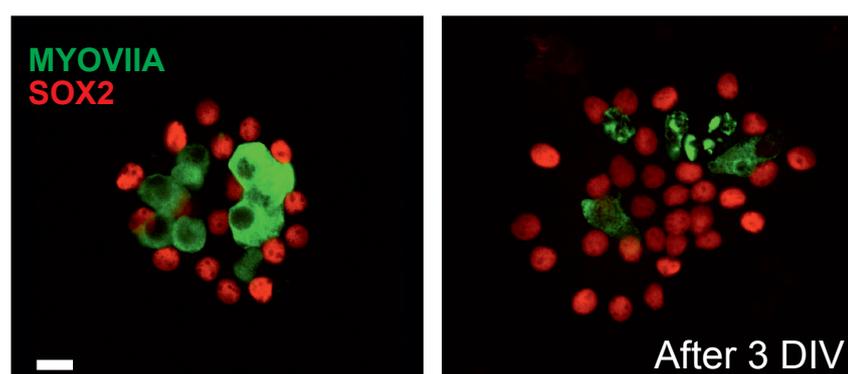


Figure 3: A representative “mini-ear” in control condition on the left with labelled supporting cells in red and hair cells in green. On the right is a mini-ear that was exposed to an ototoxic compound (Neomycin) – labelling of hair cells in green is largely diminished.

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

Driven by the fact that there is no quantitative assay for ototoxicity in humans, and that also FDA-approved drugs bear the risk to cause hearing loss, the consortium proposed to develop such assays. During the 1st funding period an *in vitro* assay for drug screening derived from murine organ of Corti progenitor cells that differentiate into “mini-ears” was designed. These “mini-ears” represent epithelial patches that include supporting and hair cell like cells mimicking as closely as possible the sensory epithelium of the *in vivo* organ equivalent (Figure 3). This method enables the generation of many hundred “mini-ears” derived from a single organ of Corti, allowing the transfer into a medium-throughput, high-content screening format. Two main project tracks were followed: Technical optimization tasks, to reach a maximal degree of automation, and the optimization of the assay to increase the quality and quantity of the “mini-ears”. The acquired knowledge was used to implement a human ototoxicity assay based on stem cells.

In the following funding periods, the consortium developed an *in vitro* platform to test for cisplatin-induced ototoxicity based on differentiated human iPSCs. This “hearing loss in a dish model” was used to test a library of known ototoxic drugs to validate the screen. Proof-of-principle screens to predict the toxicity of more than 2000 compounds (770 drugs approved by the FDA and 1280 pharmacological active compounds) were subsequently executed.

Six compounds with ototoxic properties were found by screening the compound library. Most of them were previously also detected in zebrafish-based screenings or in murine organ culture assays. Aminoglycoside antibiotics were

not detected in the screen. Two of the six identified compounds were further tested in complementary assay systems. The ototoxicity of both drugs was confirmed in well-established murine assays and human otic cells models developed from independent sources within the consortium. According to current results, false positives were not produced in our screens. Further investigations *in vitro* and *in vivo* are required before drug safety recommendations can be issued.

Drug screening aiming at otoprotection and otoregeneration

The consortium has made major progress in the design of bioassays capable of identifying compounds with otoprotective or otoregenerative activity. Otoprotective compounds prevent death of human hair cells. Otoprotective compounds may induce two different biological strategies. The first approach aims at inducing epimorphic regeneration, i.e. replacement of lost hair cells in a three-step process: dedifferentiation, proliferation and redifferentiation. The second approach termed morphallaxis, replaces lost cells by the direct phenotypic conversion of supporting cells into hair cells.

The 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 4). Due to the delay in the generation of human otic cells derived from stem cells, some of these assays were developed in mouse and others were based on human cells. These customizable *in vitro* assays were continuously improved and offer now multiple quantifiable parameters included 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

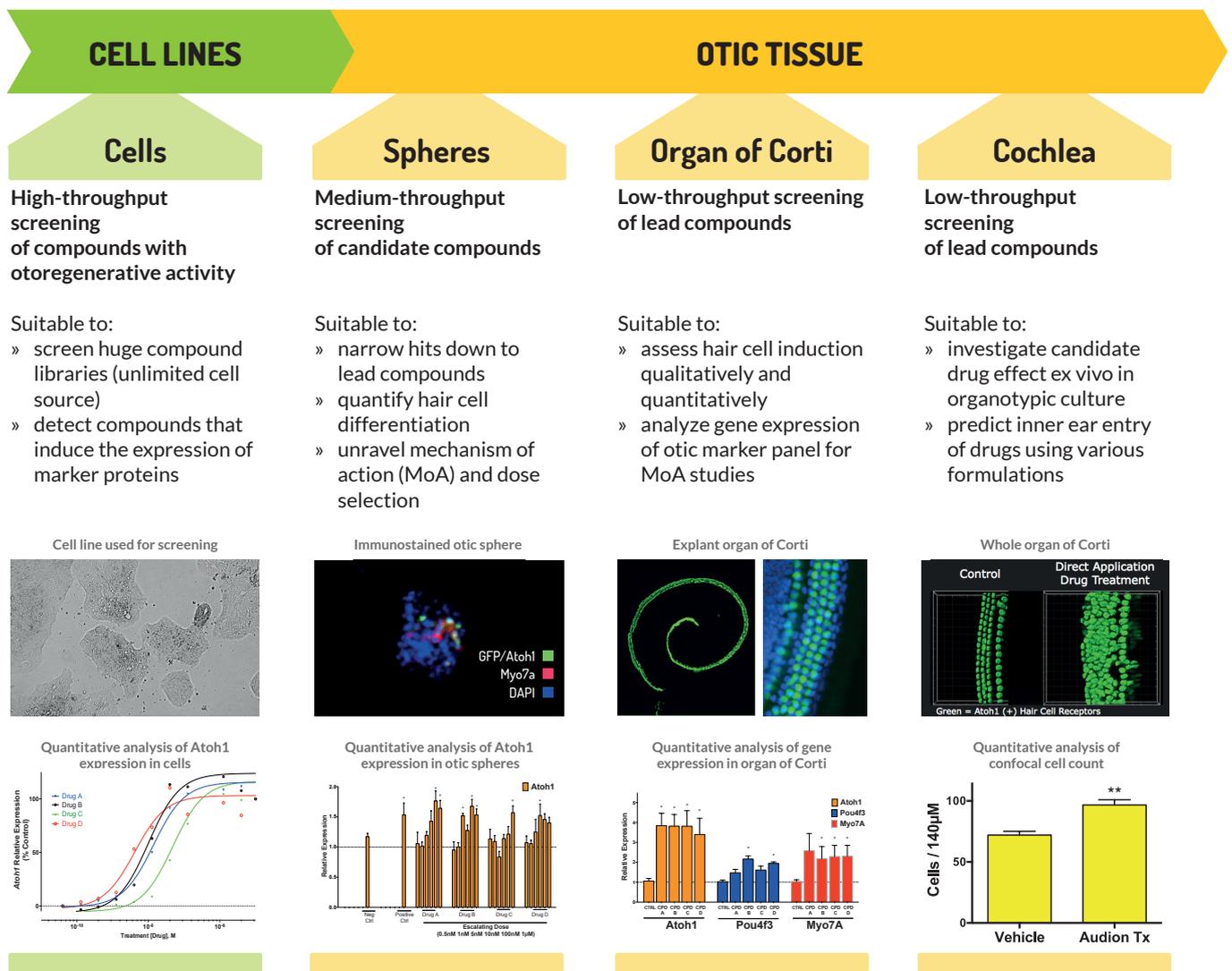


Figure 4: Screening cascade for drug discovery of OTOSTEM partner Audion Therapeutics BV. Overview of the *in vitro* otic differentiation models. Shown here is the expanded platform of models used for comparative benchmarking capabilities for compound validation studies, screening and studying phenotypic 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.

potency and specificity. The best performing compounds were validated at lower throughput in more elaborate assay systems, closer to the *in vivo* situation. The 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 such as bacterial meningitis, noise-induced hearing loss and ototoxic drug induced hearing loss due to loss of sensory hair cells or auditory neurons. The meningitis model was markedly improved and published in a high-ranking journal (Perny et al., 2016).

In the last period of the project, different surgical approaches were compared and refined to optimize the delivery of drugs or cells to the inner ear of hearing impaired animal models. Two lead compounds from the various drug discovery activities or otic cells derived from different stem cell sources and guidance protocols were tested using the improved *in vivo* models. The hearing status of these animals was quantified electrophysiologically. Improvements in functional outcomes were assessed on the cellular level by immunohistological techniques.

Compounds with otoregenerative and otoprotective activity were locally applied in *in vivo* experiments using hearing loss models (Figure 5). 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 otoregenerative 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 successfully reached clinical trial.¹

Potential impact and main dissemination activities and exploitation results

Approximately half a billion people or about 6.8% of the world's population have disabling hearing loss. The reach of hearing loss extends far beyond sensory impairment. In younger people it hampers development, and in older people an association between hearing loss and dementia is shown. The global burden of hearing loss as a result of various causes continues to grow and the costs associated with hearing loss are substantial. Hearing loss remains a major unmet

1 REGAIN project: www.regainyourhearing.eu

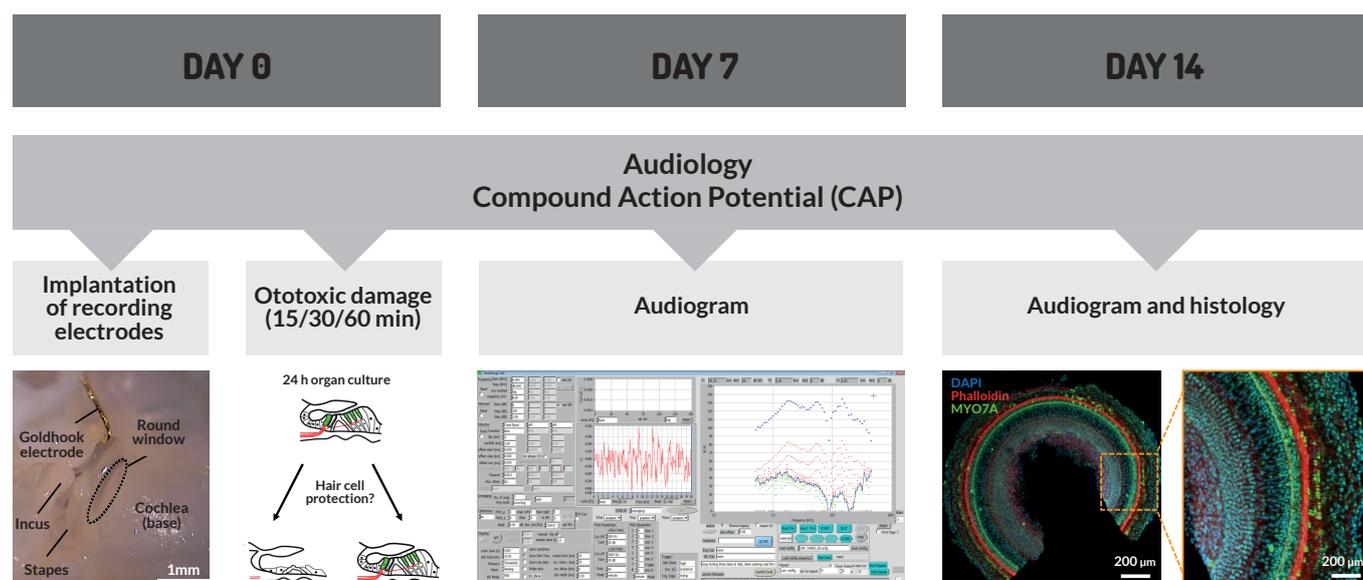


Figure 5: Schematic workflow of *in vivo* drug testing experiment to investigate otoprotective compounds of OTOSTEM partner Acousia Therapeutics GmbH. The hearing status of the animals was assessed electrophysiologically by CAP measurements before and immediately after the ototoxic insult (day 0). Otoprotective 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.

medical need as there are no pharmacological therapies. However, a number of companies have started to develop products in this field thereby focussing on otoprotection (prevent hearing loss as a result of noise and drugs) and otoregeneration (treating hearing loss after onset).

The otoprotective market is worth around 500 million USD whereas the regenerative market is expected to grow to 3.5 billion USD in 2027. Both markets are unexplored. A number of smaller companies have products in both market segments at varying stages of development in their pipeline. Most of these products are in the pre-clinical developmental stage, few products have moved on to clinical studies. This is triggering the interest of larger pharmaceutical companies which are increasingly investing in the field. The clinical success of the products in development will determine further growth of this market.

As the insight in molecular pathways leading to otoprotection and otoregeneration continues to grow, the need for good *in vitro* techniques and translational *in vivo* models increases. The OTOSTEM project addresses this need with the development of human assay systems and animal hearing loss models. Once a certain level of throughput and validation has been reached, these models can be incorporated into the drug discovery and development process to enable the identification of new and better drugs to treat hearing loss.

Although the development of human model systems has proven to be a challenge, the OTOSTEM project has generated tools that can now be used in an industrial drug discovery pipeline. Also, the knowledge that was built in the project can now successfully be used in the

academic research environment where fundamental biological mechanisms can be studied in well characterized systems with a lower throughput. Furthermore, the OTOSTEM project has generated a wealth of knowledge and experience that have significantly improved the existing non-human systems.

The OTOSTEM project has supported the two small industrial consortium partners to identify drug candidates for otoprotection, to select a drug that can be tested in the clinic for otoregeneration and to develop complementary research programs that have resulted in new grant projects.

The technologies and techniques that have been developed can be used in collaborative structures with biotechnology and pharmaceutical companies and within academia.

Overall, OTOSTEM was a successful project that has delivered new drug candidates and tools for the development of drug and cell-based therapies in the battle against hearing loss. Findings have been made available through a large number of scientific publications and presentations. Overall, 18 articles have been published in peer-reviewed journals, three articles have been submitted and several others are in preparation. All published articles are cited in the references. New publications will be posted on the OTOSTEM website at www.otostem.org. The website will remain active for another 5 years beyond the end of the project. The partners collectively presented 41 talks and 31 posters at national and international scientific events. 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

Clonal expansion of Lgr5-positive cells from mammalian cochlea and high-purity generation of sensory hair cells. Will J. McLean, Xiaolei Yin, Lin Lu, Danielle R. Lenz, Dalton McLean, Robert Langer, Jeffrey M. Karp, and Albert S.B. Edge. *Cell Reports*. 18, no. 8 (2017): 1917-1929. doi: [10.1016/j.celrep.2017.01.066](https://doi.org/10.1016/j.celrep.2017.01.066)

Role of BDNF and neurotrophic receptors in human inner ear development. Lejo Johnson Chacko, Michael J. F. Blumer, Elisabeth J. Pechriggl, Helge Rask-Andersen, Wolfgang Dietl, Alexander Haim, Helga Fritsch, Rudolf Glueckert, Jozsef Dudas, and Anneliese Schrott-Fischer. *Cell and Tissue Research*. 370, no. 3 (2017). doi: [10.1007/s00441-017-2686-9](https://doi.org/10.1007/s00441-017-2686-9)

The human “cochlear battery” – Claudin-11 barrier and ion transport proteins in the lateral wall of the cochlea. Wei Liu, Annelies Schrott-Fischer, Rudolf Glueckert, Heval Benav, and Helge Rask-Andersen. *Frontiers in Molecular Neuroscience*. 10 (2017). doi: [10.3389/fnmol.2017.00239](https://doi.org/10.3389/fnmol.2017.00239)

Molecular composition and distribution of gap junctions in the sensory epithelium of the human cochlea—a super-resolution structured illumination microscopy (SR-SIM) study. Wei Liu, Hao Li, Fredrik Edin, Johan Brännström, Rudolf Glueckert, Annelies Schrott-Fischer, Matyas Molnar, Dirk Pacholsky, Kristian Pfaller, and Helge Rask-Andersen. *Upsala Journal of Medical Sciences*. 122, no. 3 (2017): 160-170. doi: [10.1080/03009734.2017.1322645](https://doi.org/10.1080/03009734.2017.1322645)

Supernumerary human hair cells—signs of regeneration or impaired development? A field emission scanning electron microscopy study. Helge Rask-Andersen, Hao Li, Hubert Löwenheim, Marcus Müller, Kristian Pfaller, Annelies Schrott-Fischer, and Rudolf Glueckert. *Upsala Journal of Medical Sciences*. 122, no. 1 (2017): 11-19. doi: [10.1080/03009734.2016.1271843](https://doi.org/10.1080/03009734.2016.1271843)

Streptococcus pneumoniae-induced ototoxicity in organ of Corti explant cultures. Michael Perny, Magdalena Solyga, Denis Grandgirard, Marta Roccio, Stephen L. Leib, and Pascal Senn. *Hearing Research*. 350 (2017): 100-109. doi: [10.1016/j.heares.2017.04.012](https://doi.org/10.1016/j.heares.2017.04.012)

Neurosensory differentiation and innervation patterning in the human fetal vestibular end organs between the gestational weeks 8–12. Lejo Johnson Chacko, Elisabeth J. Pechriggl, Helga Fritsch, Helge Rask-Andersen, Michael J. F. Blumer, Anneliese Schrott-Fischer, and Rudolf Glueckert. *Frontiers in Neuroanatomy*. 10 (2016). doi: [10.3389/fnana.2016.00111](https://doi.org/10.3389/fnana.2016.00111)

The effect of pulsed electric fields on the electrotactic migration of human neural progenitor cells through the involvement of intracellular calcium signaling. Hisamitsu Hayashi, Fredrik Edin, Hao Li, Wei Liu, and Helge Rask-Andersen. *Brain Research*. 1652(2016): 195-203. doi: [10.1016/j.brainres.2016.09.043](https://doi.org/10.1016/j.brainres.2016.09.043)

Distinct capacity for differentiation to inner ear cell types by progenitor cells of the cochlea and vestibular organs. Will J. McLean, Dalton T. McLean, Ruth Anne Eatock, and Albert S. B. Edge. *Development*. (2016): dev.139840. doi: [10.1242/dev.139840](https://doi.org/10.1242/dev.139840)

Single-cell analysis delineates a trajectory toward the human early otic lineage. Megan Ealy, Daniel C. Ellwanger, Nina Kosaric, Andres P. Stapper, and Stefan Heller. *Proceedings of the National Academy of Sciences*. 113, no. 30 (2016): 8508-8513. doi: [10.1073/pnas.1605537113](https://doi.org/10.1073/pnas.1605537113)

The severity of infection determines the localization of damage and extent of sensorineural hearing loss in experimental pneumococcal meningitis. Michael Perny, Marta Roccio, Denis Grandgirard, Magdalena Solyga, Pascal Senn, and Stephen L. Leib. *Journal of Neuroscience*. 36, no. 29 (2016): 7740-7749. doi: [10.1523/JNEUROSCI.0554-16.2016](https://doi.org/10.1523/JNEUROSCI.0554-16.2016)

Scanning electron microscopic examination of the extracellular matrix in the decellularized mouse and human cochlea. Peter A. Santi, Robair Aldaya, Alec Brown, Shane Johnson, Tyler Stromback, Sebahattin Cureoglu, and Helge Rask-Andersen. *JARO*. 17, no. 3 (2016): 159-171. doi: [10.1007/s10162-016-0562-z](https://doi.org/10.1007/s10162-016-0562-z)

Sox2 in the differentiation of cochlear progenitor cells. Judith S. Kempfle, Jack L. Turban, and Albert S. B. Edge. *Scientific Reports*. 6 (2016): 23293. doi: [10.1038/srep23293](https://doi.org/10.1038/srep23293)

Strategy towards independent electrical stimulation from cochlear implants: Guided auditory neuron growth on topographically modified nanocrystalline diamond. Yixiao Cai, Fredrik Edin, Zhe Jin, Andrei Alexsson, Olafur Gudjonsson, Wei Liu, Helge Rask-Andersen, Mikael Karlsson, and Hao Li. *Acta Biomaterialia*. 31 (2016): 211-220. doi: [10.1016/j.actbio.2015.11.021](https://doi.org/10.1016/j.actbio.2015.11.021)

3D computational reconstruction of tissues with hollow spherical morphologies using single-cell gene expression data. Robert Durruthy-Durruthy, Assaf Gottlieb, and Stefan Heller. *Nature Protocols*. 10, no. 3 (2015): 459-474. doi: [10.1038/nprot.2015.022](https://doi.org/10.1038/nprot.2015.022)

Cell cycle reactivation of cochlear progenitor cells in neonatal Fucci mice by a GSK3 small molecule inhibitor. Marta Roccio, Stefan Hahnewald, Michael Perny, and Pascal Senn. *Scientific Reports*. 5 (2015): 17886. doi: [10.1038/srep17886](https://doi.org/10.1038/srep17886)

Inner ear hair cell-like cells from human embryonic stem cells. Mohammad Ronaghi, Marjan Nasr, Megan Ealy, Robert Durruthy-Durruthy, Joerg Waldhaus, Giovanni H. Diaz, Lydia-Marie Joubert, Kazuo Oshima, and Stefan Heller. *Stem Cells and Development*. 23, no. 11 (2014): 1275-1284. doi: [10.1089/scd.2014.0033](https://doi.org/10.1089/scd.2014.0033)

Generation of otic sensory neurons from mouse embryonic stem cells in 3D culture. Michael Perny, Ching-Chia Ting, Sonja Kleinlogel, Pascal Senn and Marta Roccio. *Frontiers in Cellular Neuroscience*. 11 (2017): 1-12. doi: [10.3389/fncel.2017.00409](https://doi.org/10.3389/fncel.2017.00409)

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